Interstate Shellfish Sanitation Conference

Task Force I Report

2013 Biennial Meeting
January 25 – January 31, 2014

Patti Fowler
Chair

John Tesvich
Vice Chair

Joe Migliore
Region 1 Regulatory

Darcie Couture
Region 1 Industry

Bruce Friedman
Region 2 Regulatory

Pete Jensen
Region 3 Industry

Dominic Guadagnoli
Region 4 Regulatory

Jerrod Davis
Region 6 Regulatory

Ken Weigardt
Region 6 Industry

Joe Jewell
Board Consultant

Joel Hansel
EPA

Bill Watkins
FDA

Calvin Walker
NOAA

The St. Anthony Riverwalk Hotel
"a national historic landmark"

Proposal Subject:	Rapid Extraction Method for PSP and ASP
Specific NSSP Guide Reference:	Section II. Model Ordinance Chapter III Laboratory @.02 Methods ISSC Constitution, ByLaws, and Procedures Procedure XVI.
Text of Proposal/ Requested Action	Procedure for Acceptance and Approval of Analytical Methods for the NSSP Marine biotoxins affect farmed and wild fish and shellfish, as well as having a deleterious effect on humans. Jellett Rapid Testing has designed and developed rugged tests for the presence of Paralytic Shellfish Poison, Amnesic Shellfish Poison and Diarrhetic Shellfish Poison (under development at the time of this submittal). To facilitate the use of these tests in the field (for aquaculturists, campers, regulatory officials, etc.), Jellett Rapid Testing has developed a "low-tech" rugged alternative to the standard AOAC method designed to extract the toxins in the field as well as the laboratory. The AOAC method requires the sample to be boiled in acid at low pH and the pH adjusted with strong acids. This requires a fully equipped laboratory and significant safety precautions. The JRT Rapid Extraction Method was designed for use in remote areas, with little sophisticated backup support, by average individuals with little training and education. It is faster, less labor-intensive and less expensive than the other available method. The rapid extraction method requires vinegar and rubbing alcohol to extract the toxins. A simple, rapid, safe method such as this would make rapid tests for marine biotoxins available in remote areas, to fishermen, aquaculturists, and regulatory officials on an instant basis. The method developed by Jellett Rapid Testing Ltd has been presented to regulatory bodies over the past several years. In cooperation with individuals, governments and those organizations, the analytical method has been refined and improved. The Rapid Extraction Method is being tested in several states and foreign countries. Publications will be forthcoming. The CONSTITUTION BY-LAWS and PROCEDURES of the INTERSTATE SHELLFISH SANITATION CONFERENCE allows the ISSC, through the Laboratory Methods Review Committee, to accept analytical methods that are sufficiently validated but are not AOAC or APHA methods. This is defined in the Constitution, PROCEDURE XVI. PROCEDURE FOR ACCEPTANCE AND APPROVAL OF ANALYTICAL METHODS FOR THE NSSP. Two possible reasons for considering a method are found in Subdivisions i and ii. Subdivision i. Meets immediate or continuing need; **Subdivision ii. Improves analytical capability under the NSSP as an alternative to other approved or accepted method(s)** Currently, only the AOAC extraction for PSP and ASP are accepted. The need for a simple safe extraction method has been expressed by regulatory agencies, governmental organizations and industry for many years. The Jellett Rapid Extraction Method is being validated over a wide geographic area to demonstrate its simplicity, reliability, precision and accuracy. As a result of demonstrations of efficacy and the need that has been expressed by industry and state agencies, the Jellett Rapid Extraction Method is presented as an alternative extraction method for PSP and ASP for the NSSP as a Type III or Type IV method.

	Please see attached additional information. Suggested wording: Section II, Chapter III Laboratory @.02 Methods C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be: (1) The current AOAC and APHA methods used in bioassay for paralytic shellfish poisoning toxins; and (2) The current APHA method used in bioassay for *Karemia breve* toxins. **(3) The Jellett Rapid Extraction Method may be used for extracting PSP and ASP toxins from Shellfish by regulatory and industry laboratories.**
Public Health Significance:	Currently, only the AOAC extraction for PSP and ASP analyses are accepted. Because of many significant constraints, in practical terms, this means that analyses can be conducted only in laboratories, and then under dangerous conditions. Acceptance of the Jellett Rapid Extraction Method for PSP and ASP would allow harvesters, processors, and regulatory agencies to screen for PSP and ASP with an accepted standardized method that provides valid useable data. The Jellett Rapid Extraction Method for PSP and ASP was developed over several years in answer to the oft-stated need for a rapid, reliable, rugged, simple and safe sample preparation method. The Jellett Rapid Extraction Method for PSP and ASP is not meant to be a definitive "Standard Method", but rather to provide a supplementary extraction method that can be used in the field as well as in the lab. Possible applications for The Jellett Rapid Extraction Method for PSP and ASP include: • as a supplement to analytical methods of screening out negative samples in shellfish regulatory labs; • as a harvest management tool at aquaculture facilities or in wild shellfish harvest areas (especially near shore areas) to supplement available methods to determine if shellfish are free of PSP or ASP and safe to harvest; • as a supplement to quality control methods for shellfish processing plants, distributors and wholesalers to ensure incoming shellfish are free of PSP and ASP toxins before processing or further distribution (this test could become part of the plant's HACCP program); • as a supplement to analytical methods for water classification for biotoxins; and • as a supplement to analytical methods for broad scale ecological monitoring. The rationale for using the Jellett Rapid Extraction Method for PSP and ASP is that the method provides a rapid, reliable, rugged, simple, safe and cost-effective extraction method (especially in low-volume laboratories) for PSP and ASP that can supplement accepted tests and substantially reduce the cost of analyses. Used in conjunction with other rapid methods, the Jellett Rapid Extraction Method for PSP and ASP will supplement regulatory agency efforts and help prevent the harvest of contaminated product. Having the ability to conduct tests using an accepted rapid extraction method will allow those processors who choose to use this test to demonstrate that they are truly controlling for PSP and ASP hazards in the harvested shellfish. The Jellett Rapid Extraction Method for PSP and ASP could contribute to building long-term databases on broader scales than a regulatory lab can afford and, by using an accepted standardized method, will provide consistent results. These databases could be supplemented with industry testing in areas where there is no testing currently. This

	would extend, augment and strengthen the current food safety system broadening and refining the food safety net by increasing the number of testing sites and generating long term data in more areas. A simple, rapid, rugged, effective, reliable, safe and cost-effective extraction method, available to all harvesters, regulators, and processors, would increase the monitoring and reduce the chance that shellfish containing ASP toxins above the regulatory limit would be harvested or marketed.
Cost Information (if available):	It is difficult to determine exact costs because many government cost models do not consider capitol costs. Both extraction methods are the same through puree step, the chemicals used in both cases are minimal, as is the cost of incidental equipment (blender, pipettes, etc.). However, a comparison of time required using the Rapid Extraction Method (Add rapid liquid; Filter) with the time required using the AOAC Extraction (Add HCL; Boil; Wait; Filter; Pour in tube; Check PH) shows a significant difference. Our experience shows that it takes about 22 minutes for this portion of the AOAC extraction while it takes less than 2 minutes to complete the Jellett Rapid Extraction Method. At a salary of $33 / hour, that is a savings of $11.00 per sample extract.
Action by 2005 LMRC	Recommended referral of Proposal 05-111 to the appropriate committee as determined by the Conference Chairman.
Action by 2005 Task Force I	Recommended adoption of the Laboratory Methods Review Committee recommendation of Proposal 05-111.
Action by 2005 General Assembly	Adopted recommendation of 2005 Task Force I.
Action by USFDA	Concurred with Conference action.
Action by 2007 LMRC	Recommended no action on Proposal 05-111. Rationale – Alternative extraction method for JRT PSP should be adopted to expand utility of the test; however there are insufficient data for acceptance at this time. The submitter will send data to the Executive Office for Conference approval.
Action by 2007 Task Force I	Recommended referral of Proposal 05-111 to an appropriate committee as determined by the Conference Chairman.
Action by 2007 General Assembly	Adopted recommendation of 2007 Task Force I.
Action by USFDA	December 20, 2007 Concurred with Conference action with the following comments and recommendations for ISSC consideration. The Conference has made considerable progress in its efforts to recognize new and developing analytical methods for the detection of indicators, pathogens, and marine toxins. Much credit goes to the Laboratory Methods Review Committee and its leadership for ensuring a scientifically defensible process for adopting analytical methods under the NSSP. At the 2007 meeting numerous analytical methods were proposed for ISSC adoption. However, many of these methods were lacking the validation and associated data needed by the Laboratory Methods Review Committee to make a final determination

	regarding their efficacy for use in the NSSP. As a result the General Assembly voted "No Action" on analytical method Proposals 05-107, 05-108, 05-109, 05-111, 05-113, and 05-114. It is FDA's understanding that the intent of the "No Action" vote was not to remove these Proposals from ISSC deliberation as "No Action" normally suggests, but rather to maintain them before the Conference pending submission of additional data for further consideration. The Voting Delegates, by requesting the Proposal submitters provide additional data to the Executive Office for methods approval consistent with Procedure XVI, clearly recognized the importance and utility of these methods and intended to maintain them before the Conference for possible adoption following additional data submission. FDA requests that the ISSC Executive Board confirm FDA's understanding of this outcome. FDA fully supports such a Conference action and encourages the Executive Office to pursue submission of additional data as necessary to move forward with acceptance of these methods.
Action by 2009 LMRC	Recommended no action on Proposal 05-111. Rationale: Requested additional information has not been submitted.
Action by 2009 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation of Proposal 05-111.
Action by 2009 General Assembly	Referred Proposal 05-111 to the Laboratory Methods Review Committee.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 05-111.
Action by 2011 LMRC	Recommended acceptance of the rapid extraction method in Proposal 05-111, specifically 70% isopropanol: 5% acetic acid 2.5:1, only for use with the Abraxis shipboard ELISA for PSP as an Emerging Method solely for use in the onboard screening dockside testing protocol in the Northeast region, including George's Bank. The Laboratory Methods Review Committee further recommends: 1. The data collected during the dockside testing study be submitted to the LMRC in the SLV Method Application Protocol within 6 months of the concurrence by FDA in the Summary of Actions. 2. The validation study conducted by the State of Maine of the Abraxis laboratory ELISA with the extraction method in Proposal 05-111 be submitted to the LMRC in the SLV Method Application Protocol within 6 months of the concurrence by FDA in the Summary of Actions. 3. No action on the requested language change in Proposal 05-111 for the Model Ordinance Section II, Chapter III Laboratory @.02 Methods. Section II, Chapter III Laboratory @.02 Methods C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be: (1) The current AOAC and APHA methods used in bioassay for paralytic shellfish poisoning toxins; and (2) The current APHA method used in bioassay for *Karenia breve* toxins. **~~(3) The Jellett Rapid Extraction Method may be used for extracting PSP and ASP toxins from Shellfish by regulatory and industry~~ ~~laboratories.~~**

Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendations on Proposal 05-111.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 05-111.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 05-111.
Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended no action on Proposal 05-111 Rationale - Proposal 05-111 is resolved by action on Proposal 13-109.
Action by 2013 Task Force I	Recommends adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 05-111.

Proposal Subject:	Thermazyme™ ACP Test
Specific NSSP Guide Reference:	NSSP Section IV Guidance Documents Chapter II. Growing Areas .11 Approved Laboratory Tests
Text of Proposal/ Requested Action	Advanced Instruments, Inc. request ISSC adoption of this method for use in the National Shellfish Sanitation Program
Public Health Significance:	Thermazyme™ ACP Test will provide the basis for determining if shellfish have been thermally processed. This test will allow decisions to be based on a rapid, quantitative method rather than sensory related methods.
Cost Information (if available):	Not available
Action by 2005 LMRC	Recommended the Conference direct the ISSC Executive Office to continue to investigate the issue of standards and pursue the development of standards and report back to the Laboratory Methods Committee with progress on the issue in six (6) months.
Action by 2005 Task Force I	Recommended adoption of the Laboratory Methods Review Committee recommendation for Proposal 05-115.
Action by 2005 General Assembly	Adopted recommendation of 2005 Task Force I.
Action by USFDA	Concurred with Conference action.
Action by 2007 LMRC	Recommended referral of Proposal 05-115 to the Executive Board for consideration for interim approval. Insufficient data at this time to approve this method under Procedure XVI. Need AP curves at 145 for 15 seconds for each type of shellfish.
Action by 2007 Task Force I	Recommended adoption of the Laboratory Methods Review Committee recommendation on Proposal 05-115.
Action by 2007 General Assembly	Adopted recommendation of 2007 Task Force I.
Action by USFDA	December 20, 2007 Concurred with Conference action.
Action by 2009 LMRC	Recommended referral of Proposal 05-115 to an appropriate Committee as determined by the Conference Chairman, to review new data as it becomes available.
Action by 2009 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 05-115.
Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force I on Proposal 05-115.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 05-115.

Action by 2011 LMRC	Recommends referral of Proposal 05-115 to the appropriate committee as determined by the Conference Chairman to continue the validation of the Thermazyme ACP Test for possible use in the NSSP. LMRC further recommends the information requested by the testing lab and Advanced Instruments for validation be submitted within 6 months to be considered as an emerging method.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 05-115.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 05-115.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 05-115.
Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended no action on Proposal 05-115. Rationale - There is insufficient data to determine if the method is fit for purpose within the NSSP
Action by 2013 Task Force I	Recommends adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 05-115

Proposal Subject:	Domoic Acid Test Kit
Specific NSSP Guide Reference:	Section IV. Guidance Documents, Chapter II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods.
Text of Proposal/ Requested Action	Mercury Science Inc., in collaboration with the NOAA Center for Coastal Fisheries and Habitat Research has developed a new quantitative immunoassay for the detection of domoic acid. The assay has been commercialized and is currently sold for research use as the Domoic Acid Test Kit (product # DAK-36) (Information online at http://mercuryscience.com/DA). This product underwent thorough testing by Mercury Science to define the performance characteristics of the assay prior to commercialization. In addition, the product has been independently validated in several labs in a variety of matrices. The results of these internal and external validation studies strongly suggest that the Domoic Acid Test Kit is a rapid, low-cost, and accurate method for analysis of food, water and phytoplankton samples. At this time, Mercury Science would like to submit a partially complete Method Application to the ISSC Laboratory Methods Review Committee. Please note that the Method Application at this time does not include the completed Single Lab Validation report. The DA analyses to complete Section C. Validation Criteria are currently in progress and will continue throughout the summer. My laboratory has just received funding from the North Pacific Research Board and will be running ISSC Single Laboratory Validation Testing on butter clams (*Saxidomus giganteus*), blue mussels (*Mytilus edulis*), geoducks (*Panopea abrupta*), manila clams (*Venerupis japonica*), oysters (*Crassostrea virginica*) and razor clams (*Siliqua patula*) from Alaska later this summer. The NOAA CCFHR laboratory has similarly received their MERHAB funds last week and will be conducting a parallel Single Laboratory Validation study on butter clams, blue mussels, geoducks, manila clams, oysters, and razor clams from California, Oregon and Washington, oysters from North Carolina and quahogs (*Mercenaria mercenaria*) from Georges Bank, Massachusetts. The goal is to test a broad array of commercial species to ensure that matrix affects do not affect the assay. The results will be made available to the ISSC as they become available. The work to date includes 1) publishing the complete ELISA methodology and initial validation studies in the December 2008 issue of the Journal of Shellfish Research and 2) completing the first validation series using oysters from North Carolina. The technique was also independently validated by the Quinault tribe in Washington State. They ran the ELISA on razor clam samples gathered by the tribe for a year and sent duplicate samples to the Washington Department of Health HPLC for analyses and have made their results available for inclusion in this preliminary application. The purpose of this submission is to bring the new method to the attention of the committee in a manner that enables the method to be evaluated in a timely way. I am also seeking the committee's advice and guidance on the validation studies that will be conducted this coming summer by my laboratory and that of Wayne Litaker at NOAA. In the initial study using the oyster tissues I have closely followed the ISSC guidelines, but wanted to ensure that my interpretation was correct. I would therefore request the committee to review the methodology used in the initial oyster validation study to ensure the procedures used meet current requirements and that no additional data need to be gathered. If necessary, the protocol can be altered to meet the committee requirements.

	Please find in association with this cover letter a series of materials relevant to the evaluation of the Domoic Acid Test Kit by the ISSC Laboratory Methods Review Committee. These items included: • ISSC Method Application with Section A, Section B**,** and Section D completed (see below). • A pdf file containing the User Guide for the Domoic Acid Test Kit (DAK-36) that is included in the commercial product. (Also available online at: http://www.mercuryscience.com/DA User Guide 2007A.pdf) • A pdf file containing a reprint of the research paper entitled " RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF THE ALGAL TOXIN DOMOIC ACID," published in the December, 2008 issue of Journal for Shellfish Research. This paper describes correlation data comparing the Domoic Acid Test Kit versus HPLC analysis using several sample matrices. (Also available online at: http://mercuryscience.com/LitakerStewartDec2008.pdf) • An Excel file showing the results of a study done by the Quinault Indian Nation and the Washington Department of Health comparing razor clam analysis performed by the Domoic Acid Test Kit versus HPLC analysis. This independent study used samples collected over a nineteen month period and was planned and performed without any input from Mercury Science or NOAA. (also available online at: http://mercuryscience.com/QINWDOHdata.xls) • Preliminary tests using oyster spiked materials (see below) The ELISA method has been used independently in six laboratories and provided results equivalent to those obtained using HPLC, FMOC-HPLC and LC-MS. This is detailed in the Litaker et al. 2008 publication listed above. Based on the correlation studies conducted so far, I request that this method be considered for interim approval by the LMR committee until the remaining validation data can be provided over the next six months. Upon completion of the SLV, consideration for approval of the assay as a Level 4 method will be requested.
Public Health Significance:	The regulatory method for DA detection sanctioned by the Interstate Shellfish Sanitation Conference is a high performance liquid chromatography (HPLC) assay. Though accurate, these analyses are generally run by centralized state facilities with results typically not available for 3 to 14 days after the samples are collected. In more remote communities, many of which depend heavily on subsistence clam harvests, these long delays and the costs of sample analysis are causes for public health concern. The average cost of approximately $100 per sample limits the number of samples that can be analyzed (Harold Rourk, Washington State Department of Health, personal communication). Resource managers in coastal communities have expressed their desire for a cost-effective method for rapid and accurate determination of DA concentrations in shellfish and phytoplankton samples.
Cost Information (if available):	Anticipated cost is $7.00 per duplicate reaction

Proposed Specific Research Need/Problem to be Addressed:

This research focuses on the development is an accurate, rapid, cost-effective ELISA for use by environmental managers and public health officials to monitor Domoic Acid concentrations in environment samples. The regulatory method for DA detection sanctioned by the Interstate Shellfish Sanitation Conference is a high performance liquid chromatography (HPLC) assay. Though accurate, these analyses are generally run by centralized state facilities with results typically not available for 3 to 14 days after the samples are collected. In more remote communities, many of which depend heavily on subsistence clam harvests, these long delays and the costs of sample analysis are causes for public health concern. The average cost of approximately $100 per sample limits the number of samples that can be analyzed (Harold Rourk, Washington State Department of Health, personal communication). Resource managers in coastal communities have expressed their desire for a cost-effective method for rapid and accurate determination of DA concentrations in shellfish and phytoplankton samples. The high throughput capacity of the assay also allows for much faster response times when domoic acid events occur. The relatively low cost of the assay means that significantly more sampling is also possible on the same or smaller budget.

How will addressing this research support/improve the mission/role of the ISSC/NSSP/Industry? Support need with literature citations as appropriate.

This Assay will allow better protect public health and provide a rapid response capability when DA outbreaks occurs. It can also be adapted to monitoring phytoplankton samples so that toxic blooms can be identify and tracked. Toxic phytoplankton cells generally appear several weeks before the shellfish become toxic and can be used as an early warning system for when shellfish are likely to become toxic/

More detailed information on the assay and its potential uses is provided in a recently published article:
RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF THE ALGAL TOXIN DOMOIC ACID, Journal of Shellfish Research, Vol. 27, No. 5, 1301–1310, 2008.
Available online at: http://mercuryscience.com/LitakerStewartDec2008.pdf

Relative Priority Rank in Terms of Resolving Research Need:

Immediate	☐	**Important**	☐
Required	☐	**Other**	☐
Valuable	☐		

Estimated Cost: $7.00 per duplicate sample (~$200.00 for ELISA kit capable of analyzing 36 duplicate samples in 1.5 h)

Proposed Sources of Funding/Support: Grants have been awarded by NPRB and NOAA MERHAB program for the completion of the validation studies.

Time Frame Anticipated: Validation should be completed by January or February 2010.

Action by 2009 LMRC	Recommended referral of Proposal 09-105 to the appropriate committee as determined by the Conference Chairman.
Action by 2009 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 09-105.
Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force I on Proposal 09-105.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 09-105.

Action by 2011 LMRC	Recommends referral of Proposal 09-105 to the appropriate committee as determined by the Conference Chairman to await further data to be provided by Mercury Science the developer of the method to determine if the method is fit for purpose within the NSSP as a screening tool.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendations on Proposal 09-105.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 09-105.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-105.
Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended no action on Proposal 09-105. Rationale - There is insufficient data to determine if the method is fit for purpose within the NSSP
Action by 2013 Task Force I	Recommends adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 09-105.

Proposal Subject:	Saxitoxin (PSP) ELISA Kit
Specific NSSP Guide Reference:	Section IV. Guidance Documents, Chapter II Growing Areas, .11 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods Section II. Model Ordinance Chapter III. Laboratory @.02 Methods C. Biotoxin
Text of Proposal/ Requested Action	See attached ISSC Method Application Faster, easier, and/or more reliable methods are needed to satisfy the needs of the regulatory community and shellfish industry. The proposed ELISA method is a fast and easy to perform method with ready to use reagents i.e. analyst only needs to extract shellfish sample or dilute water sample before analysis. The proposed ELISA also provides a quantitative and/or semi-quantitative screening for shellfish extracts and/or water samples. This assay is part of Abraxis platform for marine toxin testing and complements the company's other offering for NSP, DSP, and ASP testing. The proposed ELISA can be used on-site (boat, dock) or established analytical laboratories.
Public Health Significance:	
Cost Information (if available):	As low as $15 per sample.
Action by 2009 LMRC	Recommended no action on Proposal 09-107. Rationale: Insufficient data.
Action by 2009 Task Force I	Recommends adoption of Laboratory Methods Review Committee recommendation on Proposal 09-107.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 09-107 with the following comments and recommendations for ISSC consideration. The Laboratory Methods Review Committee determined that Proposal 09-107 was accompanied by insufficient data necessary for the Committee to make a determination regarding the efficacy of the proposed saxitoxin test method for use under the NSSP. As a result the General Assembly voted "No Action" on the proposed analytical method. It has been FDA's observation and experience that the proposed ELISA method for saxitoxins presents itself as a reliable screening method to supplement existing NSSP tools for managing Paralytic Shellfish Poisoning (PSP). Therefore, FDA recommends the Conference pursue submission of additional data from Abraxis, LLC via the Proposal submission process to advance a thorough examination of this method for saxitoxin screening.
Action by ISSC Executive Board March 2010	The Executive Office will send a letter to the submitter of Proposal 09-107 to resubmit Proposal 09-107 Saxitoxin (PSP) Elisa Kit with additional information.
Action by 2011 Task Force I	Recommended adoption of Proposal 09-107 as an emerging method.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 09-107.

Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-107.
Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended no action on Proposal 09-107. Rationale - Action by the committee was not necessary. Requested additional action on this proposal was addressed by Proposal 13-109
Action by 2013 Task Force I	Recommends adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 09-107

Proposal Subject:	Post Harvest Processing
Specific NSSP Guide Reference:	NSSP Section II Model Ordinance Chapter IV Shellstock Growing Areas @.03 Growing Area Classification D (1) (a) (ii)
Text of Proposal/ Requested Action	D. Restricted Classification. (1) General (a) A growing area may be classified as restricted when: (i) A sanitary survey indicates a limited degree of pollution; and (ii) Levels of fecal pollution, human pathogens, or poisonous or deleterious substances are at such levels that shellstock can be made safe for human consumption by either relaying, depuration or low acid-canned food processing <u>or by other verifiable processes.</u>
Public Health Significance:	
Cost Information (if available):	
Action by 2011 Task Force I	Recommended referral of Proposal 11-100 to the appropriate committee as determined by Conference Chairman.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-100.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-100.
Action by 2013 Growing Area Classification Committee	Recommended no action on Proposal 11-100. Rationale – No details have been provided to determine what other verifiable processes could be used and added to the restricted classification.
Action by 2013 Task Force I	Recommends adoption of Growing Area Classification Committeence Committee recommendation on Proposal 11-100

Proposal Subject:	Re-opening Conditional Areas using Male-specific Coliphage after WTP Malfunction
Specific NSSP Guide Reference:	NSSP Section II Model Ordinance Chapter IV Shellstock Growing Areas @ .03 Growing Area Classification A. (5) (c) (ii)
Text of Proposal/ Requested Action	(ii) For emergency closures ~~(not applicable for conditional closures)~~ of harvest areas caused by the occurrence of raw untreated sewage or <u>partially treated sewage</u> discharged from a large community sewage collection system or wastewater treatment plant, the analytical sample results shall not exceed background levels or a level of 50 male-specific coliphage per 100 grams from shellfish samples collected no sooner than 7 days after contamination has ceased and from representative locations in each growing area potentially impacted; or
Public Health Significance:	Male-specific Coliphage (MSC) is an RNA virus of E. coli present in high numbers in raw sewage (on the order of 10^5 PFU/100gm). MSC is similarly resistant to chlorine disinfection as are norovirus and hepatitis A viruses, which are the viral pathogens of primary concern in sewage. MSC is a good surrogate or marker for these enteric viruses. Raw or partially treated sewage accidentally discharged into a growing area by sewage by-pass from pump station failures, broken sewage lines, or malfunctions at the wastewater treatment facilities represent a serious public health risk and require emergency closure of adjacent conditional growing areas. These closures are typically 21 days after the wastewater treatment system returns to normal operation. Recent work has shown that persistence of viruses in the growing waters is much lower in the summer months than in the winter months. Likewise, bio-accumulation rates and retention of enteric viruses in molluscan shellfish is much lower in the summer months than the winter months. MSC can be a useful tool for state shellfish programs to mitigate the negative effect of prolonged conditional closures due to wastewater treatment system failures. This approach is most appropriate in the late-spring and summer months to shorten these closures from 21 to 7 days.
Cost Information (if available):	The Male-specific Coliphage (MSC) Method is an inexpensive double-agar pour plate method that can be run in any state-certified microbiological laboratory. A refrigerated centrifuge capable of 9,000G is required which costs $10K to $12K (USD). Re-opening after 7 days using MSC method is optional for state shellfish control agencies.
Action by 2011 Task Force I	Recommended referral of Proposal 11-101 to the appropriate committee as determined by the Conference Chairman. To include FDA prepare and provide to the committee data collected using MSC in wastewater treatment plant and to work with the submitter in this proposal in analyzing that data.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-101.
Action by FDA February 26, 2012	FDA concurred with Conference action on Propoposal 11-101 with the following recommendations. FDA concurs with Conference action to refer Proposal 11-101 to an appropriate committee as determined by the Conference Chairperson. The intent of these Proposals is to expand the application of Male Specific Coliphage (MSC) for use in the management of conditional areas affected by raw or partially untreated sewage discharges from wastewater treatment plants (WWTP) or community sewage collection systems and for assessing the impact of WWTP discharges and/or sewerage collection system leaks in determining the size of adjacent areas for classification as conditionally restricted or conditionally approved. Presently, however, there is insufficient data from which to make sound science based decisions regarding the use of MSC as a more

	comprehensive tool for growing area management. Support for using MSC for conditional area management is based on uptake and elimination data for a single shellfish species, soft-shelled clams (Mya arenaria), impacted by effluent from a highly efficient WWTP at one geographic location over just one harvest season. Those data are not adequate to ensure the efficacy of MSC to safely manage other conditional areas for other species of shellfish, in other geographic regions, and over other seasons. Careful consideration needs to be given to the fact that a WWTP malfunction is often a consequence of adverse weather conditions, most notably excessive rainfall over short periods. Such rainfall events usually cause excessive land based runoff, carrying non-point fecal pollution to conditional areas. While MSC are generally ubiquitous in municipal wastewater, that is not the case with smaller pollution sources. For this reason MSC are inappropriate for indexing smaller sources and do not lend themselves well to managing areas subject to pollution from both WWTPs and other sources. Shellfish associated norovirus (NoV) outbreaks investigated by FDA's Gulf Coast Seafood Laboratory (GCSL) in the past several years have, in nearly all instances, shown MSC levels in shellfish below the assay's sensitivity(< 10 pfu/lOOml), while testing positive for NoV. These results indicate that the source of NoV was not from a WWTP. Though MSC appear to have utility and promise in assessing potential viral contamination in shellfish, much remains to be learned about their prevalence and ability to reliably index fecal contamination from various sources of human sewage. Several approaches for generating additional information and data needed to better define how MSC could potentially be used for growing area management and classification include: • Continued studies to examine the uptake and elimination of NoV, enterovirus, and MSC by shellfish species other than soft-shelled clams. These investigations should be conducted in multiple geographic locations representative of the country and over all seasons. • A SL V has been conducted and adopted by the ISSC for the method to enumerate SC in soft-shelled clams and oysters. A SL V is needed to demonstrate the efficacy of this or another method to enumerate MSC in other species of shellfish. • Understanding the efficiency of various wastewater treatment systems to inactivate/remove enteric viruses prior to discharge. • Continued studies to examine and compare MSC and enteric virus levels in wastewater influent and effluent, shellfish receiving waters, and shellfish. As requested by Task Force I, information is currently being compiled by FDA regarding MSC data from WWTP sampling. Those data should be available to the ISSC in March, 2012.
Action by 2013 Growing Area Classification Committee	Recommended referral of Proposal 11-101 to the appropriate committee as determined by the Conference Chairman. It was additionally recommended that a workgroup be formed to look at current MSC data and the science behind its potential use and applicability for use in the NSSP. The workgroup will organize a summit of outside experts, academia, and scientists to present current information and science on MSC. The group will meet at least quarterly and respond back to the Growing Area Classification Committee on its findings and recommendations. Recommended that the ISSC pursue funding to facilitate scheduling a summit to bring

	together experts to present the current science in the use of MSC.
Action by 2013 Task Force I	Recommends adoption of Growing Area Classification Committee recommendation on Proposal 11-101.

Proposal Subject:	Using Male-specific Coliphage as a Tool to Refine Determinations of the Size of the Areas to be Classified as Prohibited Adjacent to Each Outfall
Specific NSSP Guide Reference:	NSSP Section II Model Ordinance Chapter IV Shellstock Growing Areas @.03 Growing Area Classification E. (5)
Text of Proposal/ Requested Action	(c) An assessment of the combined impact of waste water treatment plant outfall and/or ex-filtration (leakage) from sewerage collection systems may be performed using male-specific coliphage assays on shellstock from adjacent growing areas. A male-specific coliphage standard of ≤ 50 PFU/100gm in shellfish meats may be used as the basis for the determination of the size of the adjacent area to be classified as conditionally restricted or approved.
Public Health Significance:	Male-specific Coliphage (MSC) is a RNA virus of E. coli present in high numbers in raw sewage (on the order of 10^5 PFU/100gm). MSC is similarly resistant to chlorine disinfection as are norovirus and hepatitis A viruses, which are the viral pathogens of concern in sewage. MSC is a good surrogate or marker for these enteric viruses and is a powerful tool to assess the impact on a growing area of raw, partially treated and treated sewage on adjacent growing areas. US and EU studies show that during the summer months MSC and associated pathogenic enteric viruses are at seasonal lows. Conversely, the risk of viral disease transmission is significantly higher in the winter months as evidenced by epidemiological studies as well as studies conducted using MSC and molecular detection of target pathogens. A better assessment of the risk of viral contamination at a particular location in an adjacent growing area at a particular time of year can be ascertained directly using MSC assays of the shellstock. Performing and evaluating dye studies on waste water treatment plant outfall evaluation is expensive and complicated. Difficulties assessing ex-filtration and leakage from the sewage collection system are well known. Few tools and less guidance are available to adequately assess the performance of a particular waste water treatment plant design and its operation with respect to virus removal. The advantages of using this specialty viral indicator to assess the overall impact of a municipal wastewater treatment system on a particular growing area are many. In growing areas impacted by waste water treatment systems, positive norovirus detected by molecular methods at significant levels in the shellfish are accompanied by corresponding high levels of MSC. MSC assays are a direct and straightforward method to determine the viral risk or validate traditional assessment techniques.
Cost Information (if available):	The Male-specific Coliphage (MSC) method is an inexpensive double-agar pour plate method, which can be run in any state-certified microbiological laboratory. A refrigerated centrifuge capable of 9,000G is required which costs $10K to $12K (USD). Cost savings and a higher level of public health protection may be realized using MSC assays of shellfish verses the level of effort needed to ascertain the viral risk indirectly through dye studies, 1000:1 dilution line determinations and performance evaluations.
Action by 2011 Task Force I	Recommended referral of Proposal 11-102 to the appropriate committee as determined by the Conference Chairman. To include FDA prepare and provide to the committee data collected using MSC in wastewater treatment plant and to work with the submitter in this proposal in analyzing that data.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-102.

Action by FDA February 26, 2012	FDA concurred with Conference action on Propoposal 11-102 with the following recommendations. FDA concurs with Conference action to refer Proposal 11-102 to an appropriate committee as determined by the Conference Chairperson. The intent of these Proposals is to expand the application of Male Specific Coliphage (MSC) for use in the management of conditional areas affected by raw or partially untreated sewage discharges from wastewater treatment plants (WWTP) or community sewage collection systems and for assessing the impact of WWTP discharges and/or sewerage collection system leaks in determining the size of adjacent areas for classification as conditionally restricted or conditionally approved. Presently, however, there is insufficient data from which to make sound science based decisions regarding the use of MSC as a more comprehensive tool for growing area management. Support for using MSC for conditional area management is based on uptake and elimination data for a single shellfish species, soft-shelled clams *(Mya arenaria),* impacted by effluent from a highly efficient WWTP at one geographic location over just one harvest season. Those data are not adequate to ensure the efficacy of MSC to safely manage other conditional areas for other species of shellfish, in other geographic regions, and over other seasons. Careful consideration needs to be given to the fact that a WWTP malfunction is often a consequence of adverse weather conditions, most notably excessive rainfall over short periods. Such rainfall events usually cause excessive land based runoff, carrying non-point fecal pollution to conditional areas. While MSC are generally ubiquitous in municipal wastewater, that is not the case with smaller pollution sources. For this reason MSC are inappropriate for indexing smaller sources and do not lend themselves well to managing areas subject to pollution from both WWTPs and other sources. Shellfish associated norovirus (NoV) outbreaks investigated by FDA's Gulf Coast Seafood Laboratory (GCSL) in the past several years have, in nearly all instances, shown MSC levels in shellfish below the assay's sensitivity(< 10 pfu/lOOml), while testing positive for NoV. These results indicate that the source of NoV was not from a WWTP. Though MSC appear to have utility and promise in assessing potential viral contamination in shellfish, much remains to be learned about their prevalence and ability to reliably index fecal contamination from various sources of human sewage. Several approaches for generating additional information and data needed to better define how MSC could potentially be used for growing area management and classification include: • Continued studies to examine the uptake and elimination of NoV, enterovirus, and MSC by shellfish species other than soft-shelled clams. These investigations should be conducted in multiple geographic locations representative of the country and over all seasons. • A SL V has been conducted and adopted by the ISSC for the method to enumerate SC in soft-shelled clams and oysters. A SL V is needed to demonstrate the efficacy of this or another method to enumerate MSC in other species of shellfish. • Understanding the efficiency of various wastewater treatment systems to inactivate/remove enteric viruses prior to discharge.

	• Continued studies to examine and compare MSC and enteric virus levels in wastewater influent and effluent, shellfish receiving waters, and shellfish. As requested by Task Force I, information is currently being compiled by FDA regarding MSC data from WWTP sampling. Those data should be available to the ISSC in March, 2012.
Action by 2013 Growing Area Classification Committee	Recommended referral of Proposal 11-102 to the appropriate committee as determined by the Conference Chairman. It was additionally recommended that a workgroup be formed to look at current MSC data and the science behind its potential use and applicability for use in the NSSP. The workgroup will organize a summit of outside experts, academia, and scientists to present current information and science on MSC. The group will meet at least quarterly and respond back to the Growing Area Classification Committee on its findings and recommendations. Recommended that the ISSC pursue funding to facilitate scheduling a summit to bring together experts to present the current science in the use of MSC.
Action by 2013 Task Force I	Recommends adoption of Growing Area Classification Committee action on Proposal 11-102.

Proposal Subject:	Alternative Male-specific Coliphage Meat Standard for Restricted Classification of Growing Areas Impacted by wastewater treatment plant outfall.
Specific NSSP Guide Reference:	NSSP Section II Model Ordinance Chapter IV Shellstock Growing Area @ .02 Bacteriological Standards G. – add new section (4)
Text of Proposal/ Requested Action	(4) Exception. If the Male-specific Coliphage indicator is used for supplemental process verification using an end-point meat standard of < 50PFU/100gm and existing fecal coliform testing requirements in Chapter XV .03 J. are used, then FC water quality monitoring is not required for the restricted classification of growing areas affected by point sources such as wastewater treatment plant outfall.
Public Health Significance:	Under shellfish relay, water quality requirements are not needed for the restricted classification when a contaminant reduction study is conducted and a minimum time period of two weeks is used. For depuration, the restricted classification requires water quality monitoring and standards. The reason for these upper FC limits is that FC meat indicator does not adequately reflect the viral risk and/or viral depuration kinetics. Male-specific coliphage is a viral indicator organism to be used in growing areas impacted by point source sewage contamination. MSC demonstrates significant advantages over FC alone for both the assessment of viral contamination and assessment of viral depuration kinetics. Upper FC limits were put into the NSSP to prevent shellfish with higher levels of viruses from being depurated. Several studies clearly show that conventional depuration using FC for process validation is not adequate to protect public health with respect to virus contamination in growing areas with significant wastewater treatment plant and sewage impact. Studies have also shown that viral levels in shellfish impacted by sewage and partially treated sewage detected using MSC and molecular techniques are much lower in the summer months than the winter months. Additionally, the viral depuration rate is higher in the summer with process waters >18°C. Recent studies have also shown that MSC is an appropriate viral indicator to assess viral depuration. Therefore, seasonal viral depuration using male-specific coliphage as well as FC for process verification is a superior approach to taking water samples using FC in a growing area adjacent to wastewater treatment plant outfall. Combining the bacterial indicator of FC and the viral indicator MSC for mitigation strategies that use meat scores is far more direct and effective than water quality sampling in this context.
Cost Information (if available):	The Male-specific Coliphage (MSC) method is an inexpensive double-agar pour plate method that can be run in any state-certified microbiological laboratory. A refrigerated centrifuge capable of 9,000G is required which costs $10K to $12K (USD). Significant cost savings and a higher level of public health protection may be realized using strategies such as seasonal coliphage depuration process validated using MSC and seasonal coliphage relay using MSC in contaminant reduction studies than requiring water quality limits using FC.
Action by 2011 Task Force I	Recommended referral of Proposal 11-103 to the appropriate committee as determined by the Conference Chairman.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-103.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-103.

Action by 2013 Growing Area Classification Committee	Recommended referral of Proposal 11-103 to the appropriate committee as determined by the Conference Chairman. It was additionally recommended that a workgroup be formed to look at current MSC data and the science behind its potential use and applicability for use in the NSSP. The workgroup will organize a summit of outside experts, academia, and scientists to present current information and science on MSC. The group will meet at least quarterly and respond back to the Growing Area Classification Committee on its findings and recommendations. Recommended that the ISSC pursue funding to facilitate scheduling a summit to bring together experts to present the current science in the use of MSC.
Action by 2013 Task Force I	Recommends adoption of Growing Area Classification Committee action on Proposal 11-103.

Proposal Subject:	Reeal ASP (Domoic Acid) Test Kit
Specific NSSP Guide Reference:	Section IV Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests, Table 4 - Marine Biotoxin Test Methods
Text of Proposal/ Requested Action	We request review of the validation study submission for the Reveal ASP (domoic acid) test kit and consideration of the method for approval as a Type IV marine biotoxin screening method for qualitative determination of domoic acid in shellfish. Add Reveal ASP (domoic acid) test to list of approved Type III and Type IV marine biotoxin methods.
Public Health Significance:	Amnesic shellfish poisoning is caused by the toxin domoic acid, produced by phytoplankton of the genus Pseudonitzschia. It is associated with eating contaminated oysters, clams, mussels, and other shellfish. There have been numerous outbreaks of ASP, and there is evidence that the occurrence of the phytoplankton responsible for ASP is widespread. Current methods for detection of domoic acid consist primarily of instrumental chemistry methods, which are laborious and time-consuming. Methods for rapid screening for domoic acid, in field and laboratory settings, are needed and will assist the industry and public health authorities in responding to this health concern. The Reveal ASP test is a lateral flow immunoassay designed for qualitative determination of domoic acid in shellfish at levels of 10 ppm (mg/kg) and above. The test uses minimal equipment and simple reagents, does not require specialized training, and can provide results in 20 minutes from sample receipt, including sample preparation.
Cost Information (if available):	Approximately $17.00 per test.
Action by 2011 LMRC	Recommended Proposal 11-107 be referred to the appropriate committee as determined by the Conference Chairman and further recommends the following guidance on the data needed from the submitter: • Analysis of samples with naturally incurred residues over a range of toxin concentrations. • Evaluate extraction recovery by comparison with HPLC. • Additional replicates of spiked samples of shellfish species. Eliminate theoretical data regarding dose response curve.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendations on Proposal 11-107.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-107.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-107.
Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended no action on Proposal 11-107. Rationale – This proposal is resolved by action on Proposal 13-112.
Action by 2013 Task Force I	Recommends adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 11-107

Proposal Subject:	Update Microbiology Laboratory Evaluation Checklist
Specific NSSP Guide Reference:	NSSP Section IV. Guidance Documents Chapter II. Growing Areas .12 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists Laboratory Evaluation Checklist – Microbiology
Text of Proposal/ Requested Action	Update Microbiology Laboratory Evaluation Checklist. Please find the updated Microbiology Laboratory Checklist attached - word document titled "Revised Microbiology Checklist 11-08-2010.doc". A summary of the changes is: • Renumbered checklist items to accommodate proposed additions and deletions and to better identify each checklist item. • Added, deleted or changed language for checklist items to be consistent with the PSP laboratory evaluation checklist. • Deleted the requirement for metals testing on reagent water and the inhibitory residue test for washed labware and increased the requirements for the bromothymol blue test. • Clarified and defined requirements for laboratory equipment, reagents including the bacterial quality control requirements for media productivity and method process control testing. • Update thermometer requirements to accommodate state bans on the use of mercury thermometers. • Updated the sterility check requirements for both in lab sterilized items and purchased pre-sterilized items.
Public Health Significance:	The current microbiology laboratory checklist was last revised in 2009 when the male specific coliphage method was approved and added to the checklist. Deficiencies have been identified while using the microbiology checklist in evaluation of laboratories and the microbiology checklist is inconsistent with some requirements in the PSP checklist. It is important that the checklist items and quality assurance requirements are clear and understandable. It is important that quality assurance requirements among the different laboratory evaluation checklists remain as consistent as possible since many monitoring laboratories perform multiple types of tests and are evaluated using multiple NSSP checklists; inconsistencies among the checklist cause confusion, extra expense and work for the laboratories.
Cost Information (if available):	None
Action by 2011 LMRC	Recommended Proposal 11-108 be referred to the appropriate committee as determined by the Conference Chairman.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 11-108.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-108.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-108.

Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended Proposal 11-108 be adopted with substitute updated document attached. Available upon request (20 page document)
Action by 2013 Task Force I	Recommends adoption of Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 11-108.

Proposal Subject:	Update PSP Laboratory Evaluation Checklist
Specific NSSP Guide Reference:	NSSP Section IV. Guidance Documents Chapter II. Growing Areas .12 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists-Laboratory Evaluation Checklist - PSP
Text of Proposal/ Requested Action	Update PSP Laboratory Evaluation Checklist. Please find the updated PSP Laboratory Checklist attached - word document titled "Revised PSP Cecklist 11-08-2010.doc". A summary of the changes is: • Added the checklist items for Jellett Rapid Test for PSP • Renumbered checklist items to accommodate proposed additions and deletions and to better identify each checklist item. • Added, deleted or changed language for checklist items to be consistent with the microbiology laboratory evaluation checklist including added laboratory education and experience requirements • Deleted the requirement for metals testing on reagent water • Clarified and defined requirements for laboratory equipment, reagents and the mouse bioassay method.
Public Health Significance:	The current PSP laboratory checklist was last revised in 2005. Since that time the Jellett Rapid Test has received approval and is not in the checklist. Deficiencies have been identified while using the PSP checklist in evaluation of laboratories and the PSP checklist is inconsistent with some requirements in the microbiology checklist which has more recently been revised . It is important that the checklist items and quality assurance requirements are clear and understandable. It is important that quality assurance requirements among the different laboratory evaluation checklists remain as consistent as possible since many monitoring laboratories perform multiple types of tests and are evaluated using multiple checklists; inconsistencies among the checklist cause confusion, extra expense and work for the laboratories.
Cost Information (if available):	None
Action by 2011 LMRC	Recommended Proposal 11-109 be referred to the appropriate committee as determined by the Conference Chairman.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 11-109.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-109.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-109.
Action by 2013 Laboratory Method Review & Quality Assurance Committee	Recommended Proposal 11-09 be referred to the appropriate committee as determined by the Conference Chairman.
Action by 2013 Task Force I	Recommends adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 11-109.

Proposal Subject:	Addition to the Requirements for the Authority During a Suspected Shellfish Related Outbreak
Specific NSSP Guide Reference:	NSSP Section II Model Ordinance Chapter II Risk Assessment and Risk Management @.01 Outbreaks of Shellfish-Related Illness
Key Words:	Reconditioning
Text of Proposal/ Requested Action:	J. Whenever the Molluscan shellfish products are deemed to be contaminated with a pathogen that would subject it to a recall, reconditioning of the product will be permitted as an alternative to control the hazard. Any such reconditioning process that is used must be validated to reduce the level of the pathogen in question to a level which is not reasonably likely to cause illness or alter the product to a form that is intended to be cooked.
Public Health Significance:	
Cost Information (if available):	
Action by 2011 Task Force I	Recommended referral of Proposal 11-115 to the appropriate committee as determined by the Conference Chairman.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-115.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-115.
Action by 2013 Growing Area Classification Committee	Recommended Proposal 11-115 be referred to the appropriate committee as determined by the Conference Chairman and that a workgroup be formed to further explore available options for PHP methods that could be used for reconditioning recalled product. The workgroup should determine a definition for "validated reconditioned process". The Committee further recommended that the workgroup report back to the Growing Area Classification Committee with its findings.
Action by 2013 Task Force I	Recommends adoption of Growing Area Classification Committee recommendation on Proposal 11-115.

Proposal Subject:	ASP ELISA for Determination of Domoic Acid in Molluscan Shellfish
Specific NSSP Guide Reference:	Section IV Guidance Documents, Chapter II. Growing Areas, .11 Approved National Shellfish Sanitation Program Laboratory Tests
Text of Proposal/ Requested Action	I am submitting for your consideration an ELISA method for the determination of domoic acid in molluscan shellfish. The method is a direct competitive ELISA based on HRP –conjugated polyclonal sheep antibodies, and has been developed and commercialized in collaboration with AgResearch (Hamilton, NZ) under the name of *ASP cDirect ELISA* and *ASP ELISA* by my company Biosense Laboratories AS, Bergen, Norway. The commercially available ASP ELISA kit is being produced under a strict QC/QA program, and manufactured in compliance with the written quality policy. The ASP ELISA has been subject to a single laboratory validation study in accordance with the AOAC guidelines, and the SLV performance parameters were published in J AOAC (Kleivdal *et al,* 2007a). The SLV study demonstrated that the ASP ELISA is a fully quantitative analytical method with good recovery and preceision. Furthermore, a comprehensive inter-laboratory study was organized with the aim to obtain collaborative study data on precision and accuracy on the ASP ELISA according to AOAC Collaborative Study Guidelines (Kleivdal *et al*, 2007b). This study involved 16 laboratories in 10 countries (including US laboratories), which also performed a method comparison between the ASP ELISA and LCMS and the HPLC reference method. The collaborative study data showed that the ASP ELISA is both accurate and precise between analytical laboratories, and that the sample data compared well with the analytical methods based on liquid chromatography. The collaborative study data was submitted to the AOAC for Official Method accreditation in 2005, and was approved First Action in 2006 (AOAC OMA 2006.02). The AOAC accredited ASP ELISA method was then proposed to the European Union (EU) as an alternative to the HPLC-based reference method used by the EU member states for the regulation of domoic acid levels in shellfish products intended for human consumption. The ASP ELISA was approved by the EU Central Reference Laboratory on Marine Biotoxins and the National Reference Laboratory network as an alternative method suitable for official use and implemented in EU regulations (EC 1224/2007). The ASP ELISA of Biosense has not previously been presented/submittted to the ISSC, but the method was mentioned in the 2005 ISSC Summary of Actions as a separate document "AOAC Reveiew of Biotoxin Laboratory Methods" 10-08-2004. In this document the ASP ELISA was mentioned as a method that would "supply alternatives to exisiting official methods" once it attained the AOAC official status. Through comprehensive validation studies we have demonstrated that the ASP ELISA from Biosense is accurate and precise, and a suitable alternative to analytical methods based on liquid chromatography. This has been acknowledged by the AOAC through Official Method Accreditation, leading to the approval by the European Union and implementation in the EU regulations. Based on the attached documentation, I request that the ASP ELISA is considered by the ISSC LMR Committee as an analytical method for the

	determination of domoic acid in molluscan shellfish as an alternative to the current HPLC-based method.
Public Health Significance:	While the analytical methods based on liquid chromatography is acknowledged by NSSP for the determination of domoic acid in shellfish, such methods require special facilities, expensive instrumentation, in addition to high-infrastructure laboratories and highly skilled operators. The strict method requirements allow only some specialized laboratories to operate the LC-methods, and these test laboratories are in many cases located far away from the production or processing site. The shellfish grower, fisher, processor or dispatch centre must therefore ship their samples away from their operation (*off-site testing*) and wait for several days before the results are returned. This time lag between sampling and return of sample results can cause problems – in particular when there is a rapid onset of toxicity in the harvesting area. The delayed communication of sample results, caused by the logistics of shipping samples and a low sample turnaround time at the off-site test laboratories cause loss of processed product, delays in product recalls and withdrawals. The continued practise with off-site testing and the lack of an effective HACCP system with *on-site monitoring* of shellfish toxins, may lead to future cases of late product recalls putting the public health at risk. Without an on-site ability to test for shellfish toxins, the risk based food safety management approach is limited to traditional monitoring programs and intensive end-product testing regimes being examples of *retroactive* and *reactive* countermeasures. While these countermeasures are useful, they still do not contribute to *solve* any of the *identified* problems occurring locally in shellfish harvesting areas. The development of an accessible, cost-efficient, and relatively simple ASP ELISA test kit for domoic acid, will make it possible to implement on-site testing at test facilities close to the point-of-problem. Such a *preventive* countermeasure will be a valuable risk management tool for pre-harvesting and post-harvest testing, allowing an immediate on-site response to elevated domoic acid levels in shellfish. The ASP ELISA will contribute to the empowerment of the shellfish industry, as they will be able to make sound harvesting decisions based *rapid and reliable* test results. Such preventive countermeasures will generally lead to reduced harvesting and catching of contaminated shellfish, with a lower fraction of non-compliant shellfish products released on the market for human consumption.
Cost Information (if available):	The full cost pr ASP ELISA 96-well kit is USD 500. Based on this the cost of obtaining a fully quantitative test result pr sample on a full plate is USD 13.9.
Action by 2013 Laboratory Method Review and Quality Assurance Committee	Recommended no action on Proposal 13-100. Rationale - There is insufficient data to determine if the method is fit for purpose within the NSSP
Action by 2013 Task Force I	Recommends adoption of Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-100.

Proposal Subject:	Laboratory Evaluations
Specific NSSP Guide Reference:	Model Ordinance Chapter III. Laboratory @.01 Quality Assurance.
Text of Proposal/ Requested Action	Model Ordinance Chapter III. Laboratory @.01 Quality Assurance A. NSSP Conformance Required for all laboratories supporting the NSSP. All laboratory analyses shall be performed by a laboratory found to conform or provisionally conform by the FDA Shellfish Laboratory Evaluation Officer or FDA certified State Shellfish Laboratory Evaluation Officer ~~(LEO)~~ in accordance with the requirements established under the NSSP. B. State Program ~~Requirements~~ Responsibilities. The Authority shall ~~assure~~ ensure that all samples are collected, maintained, transported, and analyzed in a manner that assures the validity of the analytical results. Accordingly the Authority shall: (1) Require laboratories to develop a written quality assurance plan that: (a) (b) (c) Describes all procedures and methods used to ~~collect, maintain, transport and~~ analyze samples; (d) (e) (f) Provides a quality assessment program to demonstrate laboratory and analyst competence. At a minimum this program ~~must~~ includes an annual internal assessment and ~~triennial onsite laboratory evaluations conducted by either FDA laboratory evaluation officers, and annual internal laboratory audits. For microbiological laboratories,~~ requires participation in a recognized ~~the~~ annual ~~FDA sponsored~~ proficiency test programs ~~is also required~~ (FDA, NELEOM, etc).~~; and~~ ~~(g) Requires corrective action for any deficiencies found in the laboratory quality assurance program~~ (2) Require~~s~~ laboratories to implement their quality assurance plan; (3) (4) ~~Require triennial or more frequent evaluations of all laboratories which conduct both microbial and marine biotoxin analyses used to officially support the state shellfish program;~~Require laboratories to participate in the laboratory evaluation process;~~and~~ (5) ~~Require a laboratory to be re-evaluated when any major changes in personnel ,workload, or facilities occur and when a laboratory is found in nonconformance.~~ Inform FDA Shellfish Laboratory Evaluation Officers and/or the State Shellfish Laboratory Evaluation Officer as appropriate of major changes in laboratory personnel, laboratory workload or laboratory facilities; and (6) Require corrective action for any deficiencies/nonconformities found in the quality assurance program, laboratory operations and laboratory performance.

C. FDA Responsibilities. The FDA will ensure that all laboratories generating data in support of the NSSP will be evaluated at a minimum frequency of once every three (3) years. ~~An FDA certified State Shellfish Laboratory Officer may evaluate laboratories in a different State under a memorandum of understanding agreement between the States and the FDA. The agreement shall be consistent with NSSP requirements.~~

(1) Evaluations will be conducted by either an FDA Shellfish Laboratory Evaluation Officer or an FDA certified State Shellfish Laboratory Evaluation Officer as appropriate. Normally the initial evaluation of a laboratory will be conducted by FDA

(2) Evaluations are generally onsite but can under certain circumstances be by desk audit (evaluation follow-up, action plan monitoring, nonconformity corrections, major changes in personnel, workload or facilities, etc.

D. Laboratory Evaluations.

(1) Laboratory status is determined by the number and types of nonconformities found in the evaluation using NSSP standardized criteria contained in the FDA Shellfish Laboratory Evaluation Checklists~~,~~ found in the Guidance documents Chapter II Growing Areas .12 Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists.

(a) Conforms. In order to achieve or maintain ~~its~~ conforms status under the NSSP, a laboratory ~~shall~~ must meet the following ~~requirements under the NSSP standardized~~ laboratory evaluation criteria:

(i) No critical nonconformities in the microbiologicall or marine biotoxin (PSP or NSP) component under evaluation have been identified using the appropriate FDA Shellfish Laboratory Evaluation Checklist, and;

(ii) Not more than twelve (12) key nonconformities ~~for~~ in the microbiological component or five (5) ~~for~~ in the ~~paralytic shellfish poisoning~~ marine biotoxin (PSP or NSP) components have been identified using the appropriate FDA Shellfish Laboratory Evaluation Checklist, and;

(iii) Not more than seventeen (17) critical, key, and other nonconformities in total in the microbiological component or nine (9) critical, key and other nonconformities in total in ~~for~~for the ~~paralytic shellfish poisoning~~ marine biotoxin (PSP or NSP) components have been identified using the appropriate FDA Shellfish Laboratory Evaluation Checklist. This number must ~~(~~not ~~to~~ exceed the numerical limits established for either the critical ~~and~~ or key criteria~~)~~; and

(iv) No repeat key nonconformities have been identified in the microbiological or marine biotoxin component under evaluation in consecutive evaluations using the appropriate FDA Shellfish Laboratory Evaluation Checklist.

(b) Provisionally Conforms. In order to ~~achieve~~ be deemed provisionally conforming ~~status~~ under the NSSP, a laboratory ~~shall~~ must meet the following ~~requirements under the NSSP standardized microbiological~~ laboratory evaluation criteria:

(i) Not more than three (3) critical nonconformities ~~for~~ in the microbiological component or two (2) ~~for~~ in the marine biotoxin (PSP or NSP) ~~paralytic shellfish poisoning~~ components have been identified using the appropriate FDA Shellfish Laboratory Evaluation Checklist, and;

(ii) Not more than twelve (12) key nonconformities ~~for~~ in the microbiological component or five (5) ~~for~~ in the marine biotoxin (PSP or NSP) ~~paralytic shellfish poisoning~~ components have been identified using the appropriate FDA Shellfish Laboratory Evaluation Checklist, and;

(iii) Not more than seventeen (17) critical, key and other nonconformities in total in the microbiological component or nine (9) critical, key and other nonconformities in total in the marine biotoxin (PSP or NSP) components have been identified using the appropriate FDA Shellfish Laboratory Evaluation Checklist. This number must not exceed the numerical limits established for either the critical or key criteria; and,

(iv) Not more than one (1) repeat ~~K~~key nonconformity has been identified in the microbiological or marine biotoxin component under evaluation in consecutive evaluations using the appropriate FDA Shellfish Laboratory Evaluation Checklist.

(c) Nonconformance. When a laboratory exceeds the following criteria, ~~the laboratory shall~~ it will be determined to be in nonconformance:

(i) More than three (3) critical nonconformities ~~for~~ in the microbiological component or two (2) ~~for paralytic shellfish poisoning~~ in the marine biotoxin (PSP or NSP) components have been identified using the appropriate FDA Shellfish Laboratory Evaluation Checklist, or;

(ii) More than twelve (12) key nonconformities ~~for~~ in the microbiological component or five (5) ~~for paralytic shellfish poisoning~~ in the marine biotoxin (PSP or NSP) components have been identified using the appropriate FDA Shellfish Laboratory Evaluation Checklist, or;

(iii) More than seventeen (17) critical, key and other nonconformities in total ~~for~~ in the microbiological component or more than nine (9) critical, key and other nonconformities in total ~~for paralytic shellfish poisoning~~ in the marine biotoxin (PSP or NSP) components have been identified using the appropriate FDA Shellfish Laboratory Evaluation Checklist, or;

(iv) One (1) or more repeat critical or two (2) or more repeat key nonconformities have been identified in consecutive evaluations in either the microbiological or marine biotoxin components using the appropriate FDA Shellfish Laboratory Evaluation Checklist.

E. Time Limit on Laboratory Status.

(1) Conforming Status. A laboratory found to be in conforming status for either the microbiological or marine biotoxin component or for both components has up to ninety (90) days to successfully correct all nonconformities noted in ~~the evaluation~~ each component evaluated or has an approved action plan in place to deal with the nonconformities noted. After this period, the laboratory's status will ~~shall~~ be downgraded to nonconforming if any key nonconformities remain to be successfully corrected. As a result, data being generated by the laboratory will ~~is~~ no longer be acceptable for use in support of the NSSP for the laboratory component in question

(2) Provisionally Conforms Status. A laboratory found to be in provisionally conforming status for either the microbiological or marine biotoxin component or for both components has up to sixty (60) days to successfully correct all nonconformities found in each provisionally conforming component evaluated or has an approved action plan in place to deal with the nonconformities noted. After this period, the laboratory will ~~shall~~ be assigned ~~a~~ the following status ~~of~~ for the laboratory component(s) in question:

(a) Conforms if all critical and key nonconformities have been successfully corrected in each provisionally conforming component evaluated;

(b) Nonconforming if any critical or key nonconformities remain to be successfully corrected in each provisionally conforming component evaluated. As a result, data being generated by the laboratory ~~is~~ will no longer be acceptable for use in support of the NSSP for the laboratory component in question.

(3) Nonconformance

(a) Upon a determination of nonconforming status in either the microbiological or marine biotoxin component or in both components the laboratory has up to thirty (30) days to demonstrate successful correction of all noconformities found. After this period, if all critical and key nonconformities have been successfully corrected, the status of the laboratory will be upgraded to conforming for the laboratory component(s) in question. However, if any critical or key nonconformities remain to be successfully corrected, the status of the laboratory for the laboratory component(s) in question will ~~shall~~ continue to be nonconforming; and as a result, data being generated by the laboratory for this/these laboratory component(s) will ~~is no longer~~ continue to be unacceptable ~~acceptable~~ for use in support of the NSSP.

(b) When a laboratory is found to be nonconforming in either the microbiological or marine biotoxin component or in both components ~~either~~ for failure to successfully implement the required corrective action, or for having repeated critical or key nonconformities in consecutive evaluations, the Authority ~~shall~~ will ensure that an action plan is developed to correct the situation in an acceptable and expeditious manner or discontinue use of the laboratory to support the NSSP.

(c) When all critical and key nonconformities have been

	successful corrected by a nonconforming laboratory~~;~~ for each laboratory component evaluated, the laboratory will be ~~evaluated~~ reevaluated either on-site or through a ~~careful review of appropriate documentation~~ thorough desk audit as determined by the FDA Shellfish Laboratory Evaluation Officer and the FDA certified State Shellfish Laboratory Evaluation Officer ~~LEO~~ if one is utilized by the State. Only a finding of fully conforming in laboratories whose data has ceased to be acceptable to the NSSP will restore its acceptability for use in the NSSP for the laboratory components in question. F. Laboratory Services for Depuration,Wet Storage and Post Harvest Processors. For any laboratory providing analytical testing services for depuration, wet storage or Post Harvest Processing (PHP) ~~the quality assurance program (e.g. water quality) including end product testing of any depuration processor,~~ initial and subsequent triennial evaluations will be required and conducted in accordance with @.01 and @.02 of this Chapter by an FDA Shellfish Laboratory Evaluation Officer or an FDA certified State Shellfish Laboratory Evaluation Officer as appropriate. It is understood that academic laboratories involved in PHP Validation or Verification have special circumstances such as extended periods of inactivity resulting from university schedules or funding constraints; however, written documentation of Quality Control practices will be required for time periods in which they are preparing for or actively participating in a PHP validation or verification. Times in which the lab is inactive can be explained with a not applicable notation. ~~• The Authority shall:~~ ~~1. Require the annual inspection of the laboratory in accordance with .01 and .02 of this Chapter; and~~ ~~2. Require the laboratory to retain its records for a minimum of the previous two (2) years.~~
Public Health Significance:	This proposal updates and clarifies the roles and responsibilities of the state and the FDA in the laboratory evaluation process. It also clarifies how laboratory status is determined and its effect on the acceptability of the data for use in the NSSP. In the National Shellfish Sanitation Program (NSSP) Model Ordinance Chapter XVI. Post Harvest Processing (PHP) it states that if a dealer elects to utilize a PHP for the purpose of making safety added labeling claims they must conduct a validation study to demonstrate the ability of the PHP to reduce the target pathogen(s) to acceptable levels. Specifics on target levels and approved methods of detection for pathogens are found in the Model Ordinance. All laboratory analysis must be performed by a laboratory that has been evaluated by FDA or an FDA certified LEO and found to "conform" or "provisionally conform" with the requirements of the National Shellfish Sanitation Program (NSSP) Model Ordinance Chapter III and supporting Guidance Documents. Results of the validation study should be submitted in the following format for review and consideration by state and federal shellfish control authorities. For validation of Vibrio vulnificus or Vibrio parahaemolyticus methods, checklist may be used as a guide.
Cost Information (if available):	NA
Action by 2013 Task Force I	Recommends adoption of Proposal 13-102 as submitted.

Proposal Subject:	Emergency Conditions Contingency Plan
Specific NSSP Guide Reference:	NSSP Guide Section II Model Ordinance Chapter IV @ .03 A. (1)
Text of Proposal/ Requested Action	Section II. Model Ordinance Chapter IV Shellstock Growing Areas @ .03 A. (1) (1) Emergency Conditions. A growing area shall be placed in the closed status under Section .03 A. (5) when pollution conditions exist which were not included in the database used to classify the area. Each state shall develop and maintain a current Emergency Conditions Contingency Plan that defines what the state considers to be pollution conditions which were not included in the database used to classify the area. If it is determined that an emergency condition or situation exists as defined in the Contingency Plan or other pollution condition that the state believes would compromise the sanitary condition of shellfish, then the growing area will be immediately (within 24 hours) placed in the closed status under ~~§~~Section .03 A. (5).
Public Health Significance:	When emergency conditions (spills, extreme meteorological events, …) occur that can result in water quality conditions that were not considered as part of the growing area's classification, decisions and actions must be taken quickly to close or not close the area. The need for quick action can make it difficult for the Authority to fully assess all factors involved and to determine if the conditions are different than those on which the classification was originally based. By developing an Emergency Conditions Contingency Plan, the Agency will have had sufficient time to develop the criteria while not under the pressure of responding to an emergency. As with other NSSP Contingency Plans (e.g. Biotoxin) , this plan may also include a description of actions that would be taken in response to the Emergency Conditions. These actions could include responses to effectively to minimize illness, a follow-up monitoring strategy and reopening criteria.
Cost Information (if available):	
Action by 2013 Task Force I	Recommends no action on Proposal 13-103. Rationale – Current language in Section II. Model Ordinance Chapter IV Shellstock Growing Areas @ .03 A. (1) is sufficient.

Proposal Subject:	Re-Opening Conditional Areas using Male-Secific Coliphage after WTP Malfunction
Specific NSSP Guide Reference:	NSSP Guide Section II. Model Ordinance Chapter IV. Shellstock Growing Areas @ .03 A. (5) (c) (ii)
Text of Proposal/ Requested Action	@ .03 Growing Area Classification A. General (5) Status of Growing Areas (c) Reopened Status. A growing area temporarily placed in the closed status as provided in (b) above, shall be returned to the open status only when: (ii) For emergency closures (not applicable for conditional closures) of harvest areas caused by the occurrence of raw untreated sewage discharged from a large community sewage collection system or wastewater treatment plant, the analytical sample results shall not exceed background levels or a level of fifty (50) male-specific coliphage per 100 grams from shellfish samples collected no sooner than seven (7) days <u>and no later than twenty-one (21) days</u> after contamination has ceased and from representative locations in each growing area potentially impacted <u>provided that water temperatures exceed 45° F</u>; or
Public Health Significance:	Raw or partially treated sewage accidentally discharged into a growing area by sewage by-pass from pump station failures, broken sewage lines, or malfunctions at the Wastewater Treatment facilities represent a serious public health risk and require emergency closure of adjacent conditional growing areas. Male-specific Coliphage (MSC) is a RNA virus of E. coli present in high numbers in raw sewage (on the order of 10^5 PFU/100gm). MSC is similarly resistant to chlorine disinfection as are norovirus and hepatitis A viruses, which are the viral pathogens of concern in sewage. MSC may be a good surrogate for enteric viruses. Recent work has shown that persistence of viruses in the growing waters is much lower in the summer months than in the winter months. Depuration rates of enteric viruses in molluscan shellfish is also faster in summer months. MSC can be a useful tool for state shellfish programs to mitigate the negative effect of prolonged conditional closures due to WTP system failures. This approach has been shown to work well in late-spring and summer months to shorten these closures from 21 to as short as 7 days. Most of the validation work developing this assay has been done using soft-shelled clams and oysters, during months when temperatures are above 50°F. Relatively little work on the use of this assay has been done using hard clams or when temperatures fall below 50°F. Until the assay has been appropriately validated for other shellfish species such as hard-shelled clams, and a sound correlation between MSC and enteric viruses of concern such as Norwalk virus over a range of temperatures, use of this assay on hard clams and in cold waters may result in unnecessarily prolonged closures not correlated with a real public health risk.

	Consider also the comments on proposal 11-102 by the FDA: *"Support for using MSC for conditional area management is based on uptake and elimination data for a single shellfish species, soft-shelled clams (Mya arenaria), impacted by effluent from a highly efficient WWTP at one geographic location over just one harvest season. Those data are not adequate to ensure the efficacy of MSC to safely manage other conditional areas for other species of shellfish, in other geographic regions, and over other seasons."* (emphasis added) and also: *"A SL V has been conducted and adopted by the ISSC for the method to enumerate SC in soft-shelled clams and oysters. A SL V is needed to demonstrate the efficacy of this or another method to enumerate MSC in other species of shellfish."* For several decades emergency closures have lasted for 21 days after the WTP system returns to normal operation. This practice was not associated with reports of illness associated with enteric viruses. Some states have investigated using the MSC assay to assist in speeding the reopening of waters following emergency closures, however persistent high levels have led some states to resist implementation of the MSC assay. Following Hurricane Sandy some states shipped shellfish despite high MSC counts and no illnesses were reported (Keith Skiles, personal communication).
Cost Information (if available):	The Male-specific Coliphage (MSC) Method is an inexpensive double-agar pour plate method, which can be run in any state-certified microbiological laboratory. A refrigerated centrifuge capable of 9,000G is required which cost $10K to $12K (US dollars). Re-opening after 7 days using MSC method is optional for the State shellfish control agency.
Action by 2013 Task Force I	Recommends no action on Proposal 13-104. Rationale – Limiting the sample collection to no later than twenty-one days could restrict SSCAs from gathering important data that could be used to evaluate the risk of further illnesses.

Proposal Subject:	Management Plans for Wastewater Treatment Plants
Specific NSSP Guide Reference:	NSSP Guide Section II Model Ordinance Chapter IV Shellstock Growing Areas @. 03 Growing Area Classification
Text of Proposal/ Requested Action	C. Conditional Classification (2) Management Plan Required. For each growing area, a written management plan shall be developed and shall include: (a) For management plans based on wastewater treatment plant function, performance standards that include: (i) Peak effluent flow, average flow, and infiltration flow; (ii) ~~Bacteriological or viral~~ Microbiological quality of the effluent; (iii) Physical and chemical quality of the effluent; (iv) Conditions which cause plant failure; (v) Plant or collection system bypasses; (vi) Design, construction, and maintenance to minimize mechanical failure, or overloading; (vii) Provisions for monitoring and inspecting the waste water treatment plant; and (viii) Establishment of an area in the prohibited classification adjacent to a wastewater treatment plant outfall in accordance with Section E. Prohibited Classification;
Public Health Significance:	This change is to make the language consistent with that proposed for Section II, Chapter IV @.03 E (5) Wastewater Discharges
Cost Information (if available):	This change does not incur any additional cost to the Authority or the industry beyond that inferred by the current wording.
Action by 2013 Task Force I	Recommends adoption of Proposal 13-105 as submitted.

Proposal Subject:	Wastewater Discharges for Addressing Viruses
Specific NSSP Guide Reference:	Section II Model Ordinance Chapter IV Shellstock Growing Areas @. 03 Growing Area Classification
Text of Proposal/ Requested Action	E. Prohibited Classification (5) Wastewater Discharges. (a) An area classified as prohibited shall be established adjacent to each sewage treatment plant outfall or any other point source outfall of public health significance. (b) The determination of the size of the area to be classified as prohibited adjacent to each outfall shall include the following minimum criteria: (i) The volume flow rate, location of discharge, performance of the wastewater treatment plant and the ~~bacteriological or viral~~ microbiological quality of the effluent; (ii) The decay rate of the contaminants of public health significance in the wastewater discharged; (iii) The wastewater's dispersion and dilution, including sufficient dilution to mitigate the impact of viruses in the effluent, and the time of waste transport to the area where shellstock may be harvested; and (iv) The location of the shellfish resources, classification of adjacent waters and identifiable landmarks or boundaries.
Public Health Significance:	Changing "bacteriological or viral" to "microbiological is a fairly innocuous change, since the only biological concerns for shellfish safety in wastewater are bacteria and viruses and all of these are microorganisms. This word change will also allow for any other emerging microbiological hazards, for example, *Cryptosporidium, Giardia, Cyclosporidium*, etc. Adding the phrase "including sufficient dilution to mitigate the impact of viruses in the effluent" in (iii) simply emphasizes in plain language the heightened current concern for viral pathogens in shellfish, which is thoroughly justified by the following facts related to enteric viral pathogens: (1) they only derive from humans and are most commonly and readily found in human sewage; (2) they are today's most prevalent pathogenic threat to shellfish consumers; (3) they are less effectively removed or inactivated by wastewater treatment and disinfection than bacteria; (4) they survive longer at cooler temperatures in environmental waters than bacteria; (5) they reside far longer in molluscan shellfish than bacteria; (6) they are not well indexed or predicted by the NSSP bacterial indicators; (7) routine monitoring for pathogens is not an effective preventative strategy; and, (8) ensuring sufficient dilution of contaminants by receiving waters is a proven, effective strategy for ensuring against enteric pathogens in molluscan shellfish, which is the entire intent of the statement in (a).

Cost Information (if available):	It is not intended that any of these wording changes require any additional testing or incur any additional cost for the Authority or the industry beyond that incurred by the current Model Ordinance wording.
Action by 2013 Task Force I	Recommends adoption of Proposal 13-106 as amended. E.Prohibited Classification (5) Wastewater Discharges. (a) An area classified as prohibited shall be established adjacent to each sewage treatment plant outfall or any other point source outfall of public health significance. (b) The determination of the size of the area to be classified as prohibited adjacent to each outfall shall include the following minimum criteria: (i) The volume flow rate, location of discharge, performance of the wastewater treatment plant and the microbiological quality of the effluent; (ii) The decay rate of the contaminants of public health significance in the wastewater discharged; (iii) The wastewater's dispersion and dilution, ~~including sufficient dilution to mitigate the impact of viruses in the effluent~~, and the time of waste transport to the area where shellstock may be harvested; and (iv) The location of the shellfish resources, classification of adjacent waters and identifiable landmarks or boundaries.

Proposal Subject:	Sources of Seed for Aquaculture
Specific NSSP Guide Reference:	NSSP Section II Model Ordinance Chapter VI Shellfish Aquaculture
Text of Proposal/ Requested Action	.03 Seed Shellstock Seed may come from any growing area, or from any growing area in any classification, provided that: A. The source of the seed is sanctioned by the Authority B. Seed from growing areas ~~or growing areas~~ in the restricted or prohibited classification have acceptable levels of poisonous or deleterious substances; and C. Seed from growing areas ~~or growing areas~~ in the prohibited classification are cultured for a minimum of ~~six (6) months~~ one month while average daily water temperatures are above 50 degrees F.
Public Health Significance:	Shellfish seed collected or cultured in certain growing areas that are in the prohibited classification have been shown through repeated sampling to be free of deleterious substances (John Mullen RI DOH, unpub. data, Rheault unpubl. data, Rice unpub. data, Leavitt unpub. data). A period of one month is typically adequate to purge viral and bacterial contaminants provided water temperatures are high enough to maintain active metabolic activity (above 60 degrees F or 15 degrees C) (Richards 1988). Once the Authority is satisfied that adequate sampling has demonstrated that the seed have "acceptable levels of deleterious substances", then a 30 day period of culture in open waters should be adequate to allow purging of bacterial and viral contaminants to ensure that public health is protected. The Authority retains the right to deny seed collection and culture in any area, or to require additional testing for deleterious substances, or to require longer periods to purge contaminants as necessary. The original intent of this section was to provide for purging of viral and bacterial contamination prior to harvest for consumption on the assumption that deleterious substances were at acceptable levels prior to moving the seed to grow out areas The six-month requirement was implemented as a short-hand way to ensure that seed were grown for at least one month when water temperatures exceeded 60 degrees F. It makes little sense to require relay times in excess of one month for seed that are typically more than six months from harvest size when shellstock relay times as short as two weeks are common.
Cost Information (if available):	This change should facilitate record keeping and documentation efforts required to ensure that seed from prohibited waters do not get harvested until bacterial and viral contamination has been purged.
Action by 2013 Task Force I	Recommends referral of Proposal 13-107 to an appropriate committee as determined by the Conference Chairman.

References Cited:

Richards, G. (1988), Microbial Purification of Shellfish: A Review of Depuration and Relaying, J. Food Protection 51(3)218-251.

Supporting Information:

RI DOH metals data
(oyster seed grown in Billington Cove Marina)
Unpublished data from Rd. Dale Leavitt
(clam seed grown in Warwick Cove Marina)

Proposal Subject: Action Levels, Tolerances and Guidance Levels for Poisonous or Deleterious Substances in Seafood

Specific NSSP Guide Reference: NSSP Section IV Guidance Documents
Chapter II Growing Areas
.05 Action Levels, Tolerances and Guidance Levels for Poisonous or Deleterious Substances in Seafood

Text of Proposal/ Requested Action

The FDA has established action levels, tolerances and guidance levels for poisonous or deleterious substances to control the levels of contaminants in human food, including seafood (FDA Federal Register, 1977; FDA, ~~1985~~2002). Action levels are established and revised according to criteria specified in the ***Code of Federal Regulations*** (21 CFR 109 and 509), and are revoked when a regulation establishing a tolerance for the same substance and use becomes effective. Action levels and tolerances represent limits at or above which FDA will take legal action to remove adulterated products, including shellfish, from the market. Action levels and tolerances are established based on the unavoidability of the poisonous or deleterious substance and do not represent permissible levels of contamination where it is avoidable. Guidance levels are used to assess the public health impact of the specified contaminant.

Table 1 lists action levels, tolerances and guidance levels established by the FDA for poisonous or deleterious substances in seafood, including shellfish.
Notices are published in the ***Federal Register*** as new action levels are established or as existing action levels are revised or revoked. Should any of these notices affect Table 1, FDA will issue an interpretation advising NSSP participants of this revision or addition.

~~**Table 1**~~

Action Levels, Tolerances and Guidance Levels for Poisonous or Deleterious Substances in Seafood

Class of Substance	Substance	Level	Food Commodity	Reference
Deleterious Substance	Aldrin/Dieldrin c	0.3 ppm	All Fish	CPG sec 575.100b
Deleterious Substance	Chlordane	0.3 ppm	All Fish	CPG sec 575.100b
Deleterious Substance	Chlordecone d	0.3 ppm	All Fish	CPG sec 575.100b
	DDT, DDE, TDE e	5.0 ppm	All Fish	CPG sec 575.100b
	Diquat g	2.0 ppm	All Fish	40 CFR 180.226
	Diquat g	20.0 ppm	Shellfish	40 CFR 180.226
	Glyphosate g	0.25 ppm	Fin Fish	40 CFR 180.364
	Glyphosate g	3.0 ppm	Shellfish	40 CFR 180.364
	Carbaryl	0.25 ppm	Oysters	40 CFR 180.169
	Endothall and its Monomethyl ester	0.1 ppm	All Fish	40 CFR 180.293
	Methyl	1.0 ppm	All Fish	CPG sec

		Mercury			540.600
		Heptachlor / Heptachlor Epoxide f	0.3 ppm	All Fish	CPG sec 575.100
		Mirex	0.1 ppm	All Fish	CPG sec 575.100
		Polychlorinated Biphenyls (PCBs)g	2.0 ppm	All Fish	21 CFR 109.30
		2,4-D g	0.1 ppm	Fish	40 CFR 180.142
		2,4-D g	1.0 ppm	~~All Fish~~Shellfish	40 CFR 180.142
	Chemotherapeutics	Chloramphenicol	No Residue	All Fish	21 CFR 530.41
	Chemotherapeutics	Clenbuterol	No Residue	All Fish	21 CFR 530.41
	Chemotherapeutics	Diethylstilbesterol (DES)	No Residue	All Fish	21 CFR 530.41
		Demetridazole	No Residue	All Fish	21 CFR 530.41
		Ipronidazole and other nitroimidazoles	No Residue	All Fish	21 CFR 530.41
		Furazolidine and other nitrofurans	No Residue	All Fish	21 CFR 530.41
		Fluoroquinolones	No Residue	All Fish	21 CFR 530.41
		Glycopeptides	No Residue	All Fish	21 CFR 530.41
	Natural Toxins	Paralytic Shellfish Poisoning (PSP) toxins	80 µg/100g	All Fish	CPG sec 540.250
	Natural Toxins	Neurotoxic Shellfish Poisoning (NSP) toxins	20 MU/100g	Clams, mussels, oysters, fresh frozen or canned	NSSP MO
	Natural Toxins	Azaspiracid Shellfish Poisoning (AZP) toxins	0.16 mg/kg	Clams, mussels, oysters, fresh frozen or canned	NSSP MO
	Natural Toxins	Diarrhetic Shellfish Poisoning (DSP) toxins	0.16 mg/kg	Clams, mussels, oysters, fresh frozen or canned	NSSP MO
	Natural Toxins	Amnesic Shellfish Poisoning (ASP) toxins	20 mg/kg	All Fish (except in the viscera of Dungeness crab where 30 mg/kg is permitted)	Compliance Program 7303.842

Note: the term "fish" refers to fresh or saltwater fin fish, crustaceans, other forms of aquatic animal life other than birds or mammals and all mollusks as defined in *21 CFR 123.3(d)*.

Footnotes for Table 1

a) Unless otherwise specified, the action levels, tolerances and other values listed apply to both the raw and processed food commodity. Procedures for sample collection and analyses are specified in Sections 420 and 450 of the ***FDA Investigations Operation Manual***; ***FDA Pesticide Analytical Manual* (PAM)** Volume I or II; ***AOAC Official Methods of Analysis***; ***APHA Recommended Procedures for the Examination of Sea Water and Shellfish***, Fourth Edition, 1970; or, peer reviewed literature for Domoic Acid (ASP) methodologies.
b) References designated as CPG represent the FDA Compliance Policy Guides and all associated numbers as they appear in appropriate sections of FDA's Compliance Policy Guides Manual.
c) The action level for aldrin and dieldrin are for residues of the pesticides individually or in combination. However, in adding amounts of aldrin and dieldrin do not count aldrin or dieldrin found at the level below 0.1 ppm for fish.
d) Previously listed as Kepone, the tradename for chlordecone.
e) The action level for DDT, TDE, and DDE are for residues of the pesticides individually or in combination. However, in adding amounts of DDT, TDE, and DDE do not count any of the three found below 0.2 ppm for fish.
f) The action level for heptachlor and heptachlor epoxide are for the pesticides individually or in combination. However, do not count heptachlor or heptachlor epoxide found below 0.1 ppm.
g) The levels published in 21 CFR and 40 CFR represent tolerances rather than guidance levels or action levels.

Public Health Significance:

"Table 1" within this guidance has been updated to be consistent with current FDA action levels, tolerances and guidance levels for poisonous or deleterious substances in seafood.

Cost Information (if available):

N/A – no cost

Action by 2013 Task Force I

Recommends adoption of proposal 13-108 as amended:

Table 1

Action Levels, Tolerances and Guidance Levels for Poisonous or Deleterious Substances in Seafood

Class of Substance	Substance	Level	Food Commodity	Reference
Deleterious Substance	Aldrin/Dieldrin c	0.3 ppm	All Fish	CPG sec 575.100b
Deleterious Substance	Chlordane	0.3 ppm	All Fish	CPG sec 575.100b
Deleterious Substance	Chlordecone d	0.3 ppm	All Fish	CPG sec 575.100b
	DDT, DDE, TDE e	5.0 ppm	All Fish	CPG sec 575.100b
	Diquat g	2.0 ppm	All Fish	40 CFR 180.226
	Diquat g	20.0 ppm	Shellfish	40 CFR

				180.226
	Glyphosate g	0.25 ppm	Fin Fish	40 CFR 180.364
	Glyphosate g	3.0 ppm	Shellfish	40 CFR 180.364
	Carbaryl	0.25 ppm	Oysters	40 CFR 180.169
	Endothall and its Monomethyl ester	0.1 ppm	All Fish	40 CFR 180.293
	Methyl Mercury	1.0 ppm	All Fish	CPG sec 540.600
	Heptachlor / Heptachlor Epoxide f	0.3 ppm	All Fish	CPG sec 575.100
	Mirex	0.1 ppm	All Fish	CPG sec 575.100
	Polychlorinated Biphenyls (PCBs)g	2.0 ppm	All Fish	21 CFR 109.30
	2,4-D g	0.1 ppm	Fish	40 CFR 180.142
	2,4-D g	1.0 ppm	Shellfish	40 CFR 180.142
Chemotherapeutics	Chloramphenicol	No Residue	All Fish	21 CFR 530.41
Chemotherapeutics	Clenbuterol	No Residue	All Fish	21 CFR 530.41
Chemotherapeutics	Diethylstilbesterol (DES)	No Residue	All Fish	21 CFR 530.41
	Demetridazole	No Residue	All Fish	21 CFR 530.41
	Ipronidazole and other nitroimidazoles	No Residue	All Fish	21 CFR 530.41
	Furazolidine and other nitrofurans	No Residue	All Fish	21 CFR 530.41
	Fluoroquinolones	No Residue	All Fish	21 CFR 530.41
	Glycopeptides	No Residue	All Fish	21 CFR 530.41
Natural Toxins	Paralytic Shellfish Poisoning (PSP) toxins	80 µg/100g	All Fish	CPG sec 540.250
Natural Toxins	Neurotoxic Shellfish Poisoning (NSP) toxins	20 MU/100g	Clams, mussels, oysters, fresh frozen or canned	NSSP MO
Natural Toxins	Azaspiracid Shellfish Poisoning (AZP) toxins	0.16 mg/kg	Clams, mussels, oysters, fresh frozen or canned	NSSP MO

Natural Toxins	Diarrhetic Shellfish Poisoning (DSP) toxins	0.16 mg/kg	Clams, mussels, oysters, fresh frozen or canned	NSSP MO
Natural Toxins	Amnesic Shellfish Poisoning (ASP) toxins	20 mg/kg	All Fish (except in the viscera of Dungeness crab where 30 mg/kg is permitted)	Compliance Program 7303.842

Note: the term "fish" refers to fresh or saltwater fin fish, crustaceans, other forms of aquatic animal life other than birds or mammals and all mollusks as defined in *21 CFR 123.3(d)*.

Footnotes for Table 1

a) Unless otherwise specified, the action levels, tolerances and other values listed apply to both the raw and processed food commodity. Procedures for sample collection and analyses are specified in Sections 420 and 450 of the ***FDA Investigations Operation Manual***; ***FDA Pesticide Analytical Manual*** **(PAM)** Volume I or II; ***AOAC Official Methods of Analysis***; ***APHA Recommended Procedures for the Examination of Sea Water and Shellfish***, Fourth Edition, 1970; or, peer reviewed literature for Domoic Acid (ASP) methodologies.
b) References designated as CPG represent the FDA Compliance Policy Guides and all associated numbers as they appear in appropriate sections of FDA's Compliance Policy Guides Manual.
c) The action level for aldrin and dieldrin are for residues of the pesticides individually or in combination. However, in adding amounts of aldrin and dieldrin do not count aldrin or dieldrin found at the level below 0.1 ppm for fish.
d) Previously listed as Kepone, the tradename for chlordecone.
e) The action level for DDT, TDE, and DDE are for residues of the pesticides individually or in combination. However, in adding amounts of DDT, TDE, and DDE do not count any of the three found below 0.2 ppm for fish.
f) The action level for heptachlor and heptachlor epoxide are for the pesticides individually or in combination. However, do not count heptachlor or heptachlor epoxide found below 0.1 ppm.
g) The levels published in 21 CFR and 40 CFR represent tolerances rather than guidance levels or action levels.

Proposal Subject:	Expanding the use of the Abraxis Shipboard ELISA for the determination of paralytic shellfish poisoning (PSP) toxins
Specific NSSP Guide Reference:	Section IV. Guidance Documents Chapter II. Growing Areas, .11 Approved NSSP Laboratory Tests, 4. Approved Limited Use Methods for Marine Biotoxin Testing
Text of Proposal/ Requested Action	This submission presents the Abraxis Shipboard ELISA for paralytic shellfish poisoning (PSP) toxins as a screening method for consideration as an NSSP Approved Limited Use Method. Currently the Abraxis Shipboard ELISA is approved for limited use in conjunction with the Jellett Rapid Extraction (mixture of rubbing alcohol and vinegar) and specifically for the onboard testing protocol. This proposal presents more data on the Abraxis test using the rapid extraction and also provides new data and comparisons of the test when AOAC extractions (boiling with hydrochloric acid) are performed. The data presented supports expanding the use of the Abraxis Shipboard ELISA to (1) allow for the rapid extraction OR the AOAC extraction method and (2) allow the kit to be used as a screening method beyond the onboard screening protocol.
Public Health Significance:	Paralytic shellfish poisoning intoxications result from the consumption of seafood (primarily bivalve molluscs) contaminated with neurotoxins known as paralytic shellfish toxins (PSTs). To protect public health, harvesting closures are implemented when toxicity exceeds the guidance level of 80 micrograms saxitoxin equivalents per 100 grams of shellfish tissue. As such, accurate screening and analytical methods are needed to monitor shellfish toxicity for making decisions regarding opening and closing shellfish growing areas accordingly. While the Abraxis Shipboard ELISA is already an NSSP Approved Limited Use Method for PSP toxicity determination, being able to use AOAC extractions with this kit would allow for the same extraction to be used with this method during screening and with the MBA as necessary for confirmation (without requiring a second extraction). Further expanding the use of the method beyond the onboard screening protocol would be beneficial as it would make the Abraxis Shipboard ELISA available for use by monitoring laboratories.
Cost Information (if available):	Each 96 well plate costs ~$500.
Action by 2013 Laboratory Method and Quality Assurance Review Committee	Recommended Proposal 13-109 be referred to an appropriate committee as determined by the Conference Chairman
Action by 2013 Task Force I	Recommends adoption of Laboratory Method and Quality Assurance Review Committee recommendation on Proposal 13-109.

Proposal Subject:	Immunoassay Method for Detection of Saxitoxin (PSP) from Shellfish
Specific NSSP Guide Reference:	NSSP Guide Section IV. Guidance Document Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests, 2. Approved Methods for Marine Biotoxin Testing and 4. Approved Limited Use Methods for Marine Biotoxin Testing.
Text of Proposal/ Requested Action	Review the validation for Saxitoxin (PSP) Microtiter Plate Test Kit by the Proposal Review Committee. Single Laboratory Validation Protocol for Method Approval attached.
Public Health Significance:	Rapid screening method can handle numerous samples and screen out negative samples so that it recudes the size of sample to be confirmed with regulatory methods such as mouse bioassay (MBA) or liquid chromatography with post-column oxidation (PCOX). This results in saving resources of the laboratories, and make the laboratories enable to provide rapid warning. References attached.
Cost Information (if available):	Approximate cost for the basic set up of the method is $3600
Action by 2013 Laboratory Method and Quality Assurance Review Committee	Recommended Proposal 13-110 be referred to the appropriate committee as determined by the Conference Chairman and direct the Executive Office send a letter to the submitter requesting additional information as requested by the Laboratory Methods Review and Quality Assurance Committee.
Action by 2013 Task Force I	Recommends adoption of Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-110.

Proposal Subject:	DSP PPIA Kit for Determination of Okadaic Acid Toxins Group (OA, DTX1, DTX2) in Molluscan Shellfish
Specific NSSP Guide Reference:	NSSP Guide Section IV Guidance Documents Chapter II Growing Areas .11 Approved NSSP Laboratory Tests: Marine Biotoxin Testing
Text of Proposal/ Requested Action	The DSP PPIA kit be approved as a Marine Biotoxin Laboratory Test Method
Public Health Significance:	Okadaid acid (OA) and its analogues, DTX1, DTX2, together with their ester forms are known as the group of OA-toxins. These toxins, lipophilic and heat stable, are produced by dinoflagellates and can be found in various species of shellfish, mainly in filter feeding bivalve molluscs. The OA-toxins group causes Diarrhoeic Shellfish Poisoning (DSP), which is characterised by symptoms such as diarrhoea, nausea, vomiting and abdominal pain. These symptoms may occur in humans shortly after consumption of contaminated bivalve molluscs such as mussels, clams, scallops or oysters. Inhibition of serine/threonine phosphoprotein phosphatases is assumed to be responsible for these toxic effects. Recently in the Pacific Northwest harvest areas, outbreaks of DSP have occurred.
Cost Information (if available):	Refer to Para D.1. of the Checklist
Action by 2013 Laboratory Method and Quality Assurance Review Committee	Recommended referral of Proposal 13-111 to an appropriate committee as determined by the Conference Chairman and direct the Executive Office send a letter to the submitter requesting additional information as provided by the Laboratory Methods Review and Quality Assurance Committee.
Action by 2013 Task Force I	Recommends adoption of the Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-111.

Proposal Subject:	Reveal 2.0 ASP
Specific NSSP Guide Reference:	NSSP Guide Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests
Text of Proposal/ Requested Action	We request review of the validation study submission for the Reveal 2.0 ASP (domoic acid) test kit and consideration of the method for approval as a screening method for qualitative determination of domoic acid in shellfish. Add Reveal ASP to Section IV. Guidance Documents, Chapter II. Growing Areas, .11 Approved NSSP Laboratory Tests.
Public Health Significance:	Amnesic shellfish poisoning is caused by the toxin domoic acid, produced by phytoplankton of the genus Pseudonitzschia. It is associated with eating contaminated oysters, clams, mussels, and other shellfish [1,2]. There have been numerous outbreaks of ASP, and there is evidence that the occurrence of the phytoplankton responsible for ASP is widespread. Current methods for detection of domoic acid consist primarily of instrumental chemistry methods, which are laborious and time-consuming. Methods for rapid screening for domoic acid, in field and laboratory settings, are needed and will assist the industry and public health authorities in responding to this health concern. The Reveal ASP test is a lateral flow immunoassay designed for qualitative determination of domoic acid in shellfish at levels of 10 ppm (mg/kg) and above. The test uses minimal equipment and simple reagents, does not require specialized training, and can provide results in 20 minutes from sample receipt, including sample preparation. 1] J. Sobel and J. Painter (2005), Illness caused by Marine Biotoxins. Clin. Infect. Dis. 4, 1290. [2] Van Dolah, Frances M. (2000), Marine algal toxins: origins, health effects, and their increased occurrence. *Environmental health perspectives* 108. Suppl 1, 133.
Cost Information (if available):	Approximately $17.00 per test. Reader based assay – approximate cost of Reader $1995.
Action by 2013 Laboratory Method and Quality Assurance Review Committee	Recommended adoption of this method as a Limited Use Method for the purpose of screening and precautionary closure for ASP and direct the Executive Office send a letter to the submitter requesting additional information as provided by the Laboratory Method Review and Quality Assurance Committee
Action by 2013 Task Force I	Recommends adoption of the Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-112 and recommends that the Conference be made aware the submitter of Proposal 13-112 is looking for samples to be used in testing

Proposal Subject:	Reveal 2.0 DSP
Specific NSSP Guide Reference:	NSSP Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests
Text of Proposal/ Requested Action	We request review of the validation study submission for the Reveal 2.0 DSP (okadaic acid group) test kit and consideration of the method for approval as a screening method for qualitative determination of okadaic acid group in shellfish. Add Reveal DSP to Section IV. Guidance Documents, Chapter II. Growing Areas, .11 Approved NSSP Laboratory Tests.
Public Health Significance:	Toxins that cause diarrhetic shellfish poisoning (DSP) include the okadaic acid (OA) group of toxins [1, 2] OA is produced by marine dinoflagellates such as *Dinophysis*, and has structural analogues referred to as the dinophysistoxins (DTXs). The U.S. Food and Drug Administration action limits are 160 ppb OA equivalents (OA, DTX1, DTX2, DTX3) in shellfish. LC-MS/MS methods [3] have been accepted as quantitative reference methods in many parts of the world. Assays facilitating more rapid determination of OA toxins with simplified procedures are needed by the shellfish industry and regulatory authorities. [1] J. Sobel and J. Painter (2005), Illness caused by Marine Biotoxins. Clin. Infect. Dis. 4, 1290. [2] Van Dolah, Frances M. (2000), Marine algal toxins: origins, health effects, and their increased occurrence. *Environmental health perspectives* 108. Suppl 1, 133**.** [3]Community Reference Laboratory for Marine biotoxins (CRLMB)., Agencia Española de Seguridad Alimentaria y Nutrición (AESAN). (2009). EU Harmonised Standard Operating Procedure for determination of OA-Group Toxins by LC-MS/MS. Version1. http://www.aesan.msps.es/en/CRLMB/web/procedimientos_crlmb/crlmb_standard_operating_procedures.shtml
Cost Information (if available):	Approximately $17.00 per test. Reader based assay – approximate cost of Reader $1995.
Action by 2013 Laboratory Method and Quality Assurance Review Committee	Recommended Proposal 13-113 be referred to an appropriate committee as determined by the Conference Chairman and await data to determine if the method is fit for purpose within the NSSP.
Action by 2013 Task Force I	Recommends adoption of Laboratory Method Review Committee reccomendation on Proposal 13-113.

Proposal Subject:	Receptor Binding Assay (RBA) for Paralytic Shellfish Poisoning (PSP) Toxicity Determination
Specific NSSP Guide Reference:	NSSP Guide Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests, 4. Approved Limited Use Methods for Marine Biotoxin Testing
Text of Proposal/ Requested Action	This submission presents the 'Receptor Binding Assay (RBA) for Paralytic Shellfish Poisoning (PSP) Toxicity Determination' for consideration as an NSSP Approved Limited Use Method. The RBA is a competition-based assay that employs radiolabeled saxitoxin (^{3}H-STX) to compete with PSP toxins present in standards/samples for binding sites on natural receptors in the assay. Following incubation with the receptors, unbound ^{3}H-STX is removed and the remaining labeled toxin is measured with a scintillation counter. The amount of remaining ^{3}H-STX is inversely proportional to standard/sample toxicity. The RBA offers a high-throughput, sensitive, and quantitative alternative to the mouse bioassay (MBA), which has been the long-standing reference method for PSP toxicity. Further, the RBA eliminates the use of live animals for detection of these toxins. While the RBA still uses receptors prepared from animals, the number of animals required for analysis is significantly reduced. Using native receptors as the analytical recognition elements for the assay allows for a composite measure of overall toxicity, as opposed to toxin concentrations measured by liquid chromatographic methods that require conversion factors of equivalent toxicity to calculate the overall toxicity. The RBA has undergone AOAC single- and multi-laboratory validation and is designated through AOAC as an Official Method of Analysis (OMA 2011.27). Results from those studies, and additional data, are included in this proposal submission for the RBA to be considered for approval as an NSSP Approved Limited Use Method for Marine Biotoxin Testing.
Public Health Significance:	Paralytic shellfish poisoning intoxications result from the consumption of seafood (primarily bivalve molluscs) contaminated with neurotoxins known as paralytic shellfish toxins (PSTs). This suite of toxins binds to voltage-gated sodium channels and may result in paralysis if enough toxin is consumed. In extreme cases when respiratory support is not available to the patient, the intoxication may prove fatal. Since the toxins cannot be destroyed during cooking and there is no way to remove the toxins from seafood, the best control strategy is to ensure that contaminated product never reaches the market. To protect public health, harvesting closures are implemented when toxicity exceeds the guidance level of 80 micrograms saxitoxin equivalents per 100 grams of shellfish tissue. As such, accurate analytical methods are needed to monitor shellfish toxicity for making decisions regarding opening and closing shellfish growing areas accordingly. Acceptance of the RBA as an NSSP Approved Limited Use Method for PSP toxicity determination would provide monitoring and management programs with an additional tool that can be used for monitoring toxin levels and making regulatory decisions. Not only does the RBA eliminate the need for live animals for PSP testing, it is also more sensitive than the MBA, thereby providing an early warning system for monitoring programs as toxin levels begin to rise.
Cost Information (if available):	The estimated cost for a full 96-well plate assay is ~$95.00. Including standards and samples with triplicate measurements (as well as three dilutions per sample to ensure the unknown samples fall within linear range of assay), the cost per sample for quantitative results would be ~$13.60. If running multiple plates or in

	screening mode, sample costs would be reduced. Further, the filter plates used in the RBA differ from ELISA plates in that all reagents are added to each well as needed rather than already being a component of the plate, making it more practical and cost-effective to analyze samples when there is less than a full plate.
Action by 2013 Laboratory Method and Quality Assurance Review Committee	1) Recommended approval of this method as an alternative to the mouse bioassay for PSP in mussels 2) Recommended approval of this method for Limited Use for clams and scallops for the purpose of screening and precautionary closure for PSP 3) Recommended referral of this proposal to the appropriate committee as determined by the Conference Chairman to address this method in oysters 4) Recommended Executive Office send a letter to submitter to request a checklist for evaluation of labs using this method with said checklist to be submitted within 3 months
Action by 2013 Task Force I	Recommends adoption of Laboratory Method Review and Quality Assurance Committee recommendations on Proposal 13-114.

Proposal Subject:	PSP HPLC-PCOX Method Evaluation Checklist
Specific NSSP Guide Reference:	2011 NSSP Section IV. Guidance Documents Chapter II. Growing Areas .12 Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers including Laboratory Evaluation Checklist-Laboratory Checklist-PSP
Text of Proposal/ Requested Action	Establish a PSP Laboratory Evaluation Checklist for the HPLC-PCOX method. Please find the HPLC-PCOX checklist attached-word document titled "PSP HPLC PCOX checklist.docx" There is no summary of changes as no previous checklist exists for this procedure
Public Health Significance:	The HPLC-PCOX method has been an approved limited use method since 2009, yet no checklist exists to allow evaluation of laboratories who utilize this method. Use of this method provides states much more detailed toxin profiles as well as helping eliminate animal testing. It is important that the checklist items and quality assurance requirements are clear and understandable.
Cost Information (if available):	For laboratories that do not already possess a HPLC post column reaction system, the upfront cost can be significant. Once in place, the costs per test are not significantly different than that imposed by the capital cost of the mouse bioassay.
Action by 2013 Laboratory Method and Quality Assurance Review Committee	Recommended Proposal 13-115 be referred to an appropriate committee as determined by the Conference Chairman
Action by 2013 Task Force I	Recommends adoption of the Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-115.

Proposal Subject:	Shellfish Quarantine Guidance Document
Specific NSSP Guide Reference:	NSSP Guide Section II. Model Ordinance Chapter IV. @.04 A. (4) Section IV. Guidance Documents Chapter II. Growing Areas .02 Guidance for Developing Marine Biotoxin Contingency Plans
Text of Proposal/ Requested Action	Chapter IV, @.04 A (4) describes agreements or memoranda of understanding between the Authority and individual shellfish harvesters or individual shellfish dealers, to allow harvesting during marine biotoxin closures under specific, controlled conditions. The state of Florida has successfully implemented such an agreement to address Neurotoxic Shellfish Poisoning (NSP) for over a decade. This pilot project, developed in consultation with FDA, has resulted in zero cases of NSP in commercially harvested shellfish from Florida waters. NSP may affect any Gulf or South Atlantic state and therefore Florida wishes to provide ISSC member states with a proven quarantine protocol template for incorporation into the Model Ordinance Section IV. Guidance Documents. Chapter II. Growing Areas .02 Guidance for Developing Marine Biotoxin Contingency Plans. Text of the proposed guidance is as follows: Example Protocol For Quarantine Harvest of Shellfish From Aquaculture Leases During *Karenia brevis* Closures: A. Closure of an entire shellfish growing area due to *Karenia brevis* shall be in accordance with Model Ordinance Chapter IV @.04 C (1). B. When a shellfish growing area is closed due to *Karenia brevis*, the Authority may allow harvest of shellfish from selected aquaculture leases within a specific zone by authorized harvesters and subsequent controlled quarantine at a certified shucker packer or shellstock shipper. This option would not be available if any Authority-collected water samples in the specific zone exceeded 200,000 cells per liter of *Karenia brevis*. Zone is defined as an Authority-delineated geographic area within a Conditionally Approved or Approved classified shellfish growing area. *Controlled quarantine conditions*: The Authority will determine and plot the specific zones. Certified processors possessing a valid shellfish processing plant certification license must have written permission from the Authority to engage in this activity. To be eligible for participation in the quarantine program, the certified processor must: (1) Provide the Authority with written and signed agreements the processor has with shellfish aquaculture leaseholders who would be supplying the shellfish and; (2) Notate on their application letter which FDA-approved marine biotoxin laboratory will be used to conduct the approved mouse bioassay and; (3) Provide the Authority with the cooler capacity, physical address and current certification number of the facility to be used for controlled quarantine of shellfish. All quarantine coolers must be non-mobile, secure from unauthorized access and equipped with warning signs in a language readily understood by all employees. Participation in each week's quarantine program is only possible for certified processors who:

(1) have written permission on file with the Authority and are on an Authority-controlled document listing current approved quarantine program processors and;

(2) possess emailed permission granted by the Authority the day before harvest for that one specific quarantine and;

(3) propose harvesting a quantity of shellfish that meets the Authority-established minimum number but does not exceed the maximum allowed number of shellfish of one specific species for that day.

Under no circumstances may any approved processor participate in any quarantine until they possess written (emailed) documentation sent by the Authority before each specific quarantine event.

- The authorization email sent by the Authority shall explicitly state the permissible species that may be harvested by that approved processor.
- The Authority will notify the appropriate law enforcement entity in charge of patrol of shellfish growing areas with a list of participants in that specific day's harvest.
- Persons harvesting a species not authorized for that day's harvest will be subject to seizure of that harvest by the Authority. In addition, the Authority will immediately seize and destroy product which is improperly tagged, violates any National Shellfish Sanitation Program (NSSP) Model Ordinance regulations, state laws or is from non-authorized participants.
- Co-mingling of species is not allowed to make up an individual lot.

Violation of the terms of this protocol may result in the termination of the participant's future eligibility in the quarantine program, as determined by the Authority.

Prior to being considered for participation in any specific quarantine event, approved processors shall be contacted by the Authority and asked to provide the name of the species they plan to harvest and the quantity they plan on harvesting. Quantities shall be described as approximate total number by species in addition to total number of baskets, containers, bags, etc. with specific weights (if applicable) for those baskets, containers, bags, etc.

Eligible processors should be aware that daily implementation of this program is contingent on marine biotoxin laboratory availability as well as Authority staffing considerations given staff time necessary to fulfill the requirements of the program.

Regulatory considerations on behalf of the Authority and staffing considerations on behalf of the marine biotoxin lab necessitate an Authority-developed maximum number of samples that could be potentially tested on any given week.

The Authority may implement a lottery, random rotation or similar procedure to ensure a fair distribution of testing opportunities among the

eligible processors. It is suggested that the Authority develop this procedure with industry involvement.

Once specific permission is received from the Authority, the processor:

(1) may receive properly tagged shellfish from eligible aquaculturists only as indicated in the Authority's authorization email;
(2) must upon receipt of shellfish, separate and maintain the shellfish into specific lots [A Lot is defined as shellfish of one species from no more than one day's harvest from a specific zone within a shellfish growing area];
(3) must place shellfish under proper controls and quarantine; Proper controls and quarantine are defined by bold, clear, warning signage signaling the properly tagged and segregated shellfish within the processor's cooler are under quarantine and must not be moved until Authority permission is obtained pending outcome of laboratory testing. The signage should be such that it is clear to anyone entering the cooler (including facility employees and/or regulatory inspectors) that the affected shellfish are under quarantine. Wrapping of the entire lot with a single bright red or yellow ribbon or equivalent attached to the bold warning sign will further reinforce the warning message.
(4) must allow the Authority to take 2 random samples [minimum of 20 shellfish per each sample] from each lot and deliver to the approved laboratory for approved mouse bioassay;
(5) must hold all shellfish in quarantine at the approved processor's certified facility until receiving official written test result notice from the Authority via email or fax that the shellfish are cleared for sale;
(6) must either return shellfish to aquaculture lease(s) in the zone(s) from where harvested if any sample in a lot is 20 Mouse Units / 100 grams or greater or destroy the shellfish, both activities of which must be witnessed and documented by the Authority;
(7) may release the shellfish in a lot to the market if both samples from that lot are <20 Mouse Units / 100 grams;
(8) must cease this activity if any Authority collected red tide cell counts in the specific zone exceeds 200,000 cells per liter of *Karenia brevis;*, and
(9) must document all of the requirements listed above in the approved facility HACCP plan.

C. If cell counts in all water samples fall to 5,000 cells/L or less *Karenia brevis* in the entire area, the Authority will collect shellfish meat samples for toxicity testing and the entire Shellfish Harvesting Area will be reopened if results of all samples are <20 MU/100g.

I _____(print name) have received a copy of this quarantine protocol and I agree to abide by all terms and conditions. I understand I am bound by the terms of this agreement during the period of time that I am processing shellfish from a shellfish growing area that is currently in the closed status due to *Karenia brevis*.

_____

_____

Signed _____ Date

Public Health Significance:	Closures of shellfish growing areas due to Neurotoxic Shellfish Poisoning (NSP) may occur at any time in the Gulf of Mexico and to a lesser degree, the Atlantic coast. Well established procedures for detecting and responding to *Karenia brevis* blooms have safeguarded public health. Clear early warning signs, a cell count action level with a high factor of safety and established sampling networks provide excellent public health protection. A very real impact of *Karenia brevis* blooms is the resulting long-term closures of shellfish growing areas and severe economic impact to commercial shellfish operations. Florida addressed this issue after studying years of water quality samples and mouse bioassay results from shellfish growing areas. Hydrodynamic studies linked to water samples obtained from fixed stations over an extended period of time established clear patterns in distribution of *Karenia brevis*. Working in conjunction with harmful algal bloom researchers, shellfish growing area managers, FDA and industry, Florida developed a NSP quarantine protocol that has resulted in the retention of a shellfish industry in one of the most severely impacted HAB regions of the Gulf while protecting public health as required by the Model Ordinance. An enormous amount of data has been generated and reviewed during the years this protocol has been used. Repeated mouse bioassay testing on shellfish exposed to different levels of *Karenia brevis* has provided Florida with sufficient data to refine the protocol into a powerful management tool. Florida's experience pre-quarantine protocol was unfortunate, as several fledgling businesses failed due to repeated NSP closures. It was this economic damage that spurred the aforementioned collaborative effort between leading edge HAB researchers, shellfish growing area managers, FDA and industry. If adopted, shellfish producing states impacted by *Karenia brevis* could reference this protocol in the Guidance Document and use it to effectively manage NSP closures.
Cost Information (if available):	
Action by 2013 Task Force I	Recommends referral of Proposal 13-116 to an appropriate committee as determined by the Conference Chairman.

Proposal Subject:	Certification of State Shellfish Laboratory Evaluation Officers
Specific NSSP Guide Reference:	NSSP Guide Section IV Guidance Documents Chapter II. Growing Areas .12 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists
Text of Proposal/ Requested Action	Laboratory results from the ~~bacteriological~~ microbiological and marine biotoxin testing of shellfish and shellfish growing waters ~~and meats~~ are widely used in the National Shellfish Sanitation Program (NSSP) to aid in determining the safety of shellfish for human consumption. Experience with the ~~bacteriological~~ microbiological and marine biotoxin analyses of shellfish and shellfish growing waters have indicated that minor differences in laboratory procedures or techniques might cause wide variations in the results. ~~Improper handling of the sample may also cause variations in results during collection or transportation to the laboratory~~. To ensure uniformity ~~nationwide~~ NSSP wide in the application of standards for shellfish and shellfish growing waters, a comprehensive, effective laboratory quality assurance (QA) program is necessary to ~~substantiate~~ demonstrate the validity of analytical results. ~~A~~ The laboratory ~~quality assurance~~ QA program is the systematic application of the practices essential to remove or minimize errors that may occur in any laboratory operation caused by personnel, ~~apparatus,~~ equipment, media, reagents~~, sampling procedures,~~ and analytical methodology. ~~(APHA, 1985).~~ Integral to laboratory quality assurance is a strong program for the external assessment or evaluation of laboratory performance. The laboratory evaluation process has evolved over the years to accommodate changes in microbiology and marine biotoxin procedures brought about by NSSP Workshops and more recently by the Interstate Shellfish Sanitation Conference (ISSC). In 1985, FDA issued an interpretation entitled "Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers" (SS#35). This Interpretation allowed NSSP laboratories which had been previously evaluated by FDA Shellfish Laboratory Evaluation Officers to be subsequently evaluated by qualified state personnel as certified State Shellfish Laboratory Evaluation Officers. This guidance describes the procedure for the certification of these individuals as State Shellfish Laboratory Evaluation Officers. ~~Requirements for evaluating laboratories that analyze samples under the NSSP have increased significantly since the 1970's. The number of laboratories participating in the shellfish program has also increased. Several states now have multiple laboratories that provide these analyses. Some states have officially designated city, county or private laboratories to conduct analyses supporting their shellfish sanitation programs. Some states are also authorizing the use of private laboratories to monitor depuration operations. More states are maintaining a marine biotoxin analytical capability in their laboratories; and more foreign laboratories are involved in the NSSP. Historically, FDA has evaluated all these laboratories. Reduction in FDA staffing has made it difficult to evaluate the many state, county, municipal, and foreign shellfish laboratories operating in support of the NSSP. If states with multiple laboratory support would exercise their option to accept responsibility for evaluating their laboratories by employing a State Shellfish Laboratory Evaluation Officer (State Shellfish LEO), FDA would be able to better meet its NSSP responsibilities.~~ General Provisions 1. If the State Shellfish Control Authority (Authority) uses the analytical

services of private/commercial/fee for services laboratories to support the NSSP, then he/she should select a qualified individual to become certified as a State Shellfish Laboratory Evaluation Officer (State Shellfish LEO).

2. If the Authority uses the analytical services of multiple public laboratories (state, county, parish town, etc.) to support the NSSP, then he/she may select a qualified individual to become a State Shellfish LEO.
3. If the Authority chooses not to participate in the certification process, FDA can evaluate the state's public laboratories. FDA, however, does not normally evaluate private/commercial/fee for services laboratories. FDA may, under certain circumstances as resources permit, evaluate these laboratories on a case-by-case basis at the request of the Authority. This request must be in writing and made through the FDA Regional Shellfish Specialist.
4. State Shellfish LEOs will perform official NSSP evaluations of laboratories which have been previously evaluated by FDA and been found to fully conform to NSSP laboratory requirements.
5. State Shellfish LEOs may evaluate laboratories in a different state under a memorandum of understanding between the states involved and FDA consistent with NSSP requirements.
6. State Shellfish LEOs may not evaluate laboratories in which they are employed or which they supervise or laboratories within the same supervisory chain of command to ensure complete objectivity in the evaluation process and avoid the appearance of a conflict of interest.
7. To qualify for certification, the prospective State Shellfish LEO should be:
    a. A state employee;
    b. Have shellfish laboratory experience or a laboratory background;
    c. Preferably have laboratory evaluation experience; and,
    d. Be free from any commercial, financial or other pressures or conflicts of interest that might cause or appear to cause the prospective State Shellfish LEO to act in other than an impartial or non-discriminatory manner.
8. If the prospective or current State Shellfish LEO is employed by the laboratory supporting the NSSP, that laboratory must be fully conforming to NSSP requirements or the individual will not be certified and if currently certified, certification will be revoked.

Responsibilities of the State Shellfish Control Authority

1. The Authority must ensure that appropriate written documentation is provided to FDA to demonstrate that a prospective State Shellfish LEO is adequately qualified to assume the responsibilities of a State Shellfish LEO as described above.
2. The Authority must provide or ensure that adequate time, resources and support are made available to the State Shellfish LEO to fully participate in the certification process and to fulfill his/her obligation as a State Shellfish LEO.

FDA's Responsibilities

1. FDA is responsible for the certification/recertification of State Shellfish LEOs.
2. As a result FDA must:
    a. Select qualified individuals to receive training based upon the documentation supplied by the Authority;
    b. Develop and provide training that will enable prospective and current

State Shellfish LEOs to consistently and uniformly apply evaluation criteria in determining the competence of laboratories to support or continue to support the NSSP;

c. Certify prospective State Shellfish LEOs that successfully complete the certification process;

d. Maintain communication with State Shellfish LEOs as needed to provide guidance and updates relevant to the NSSP laboratory evaluation program;

e. Recertify current State Shellfish LEOs pursuant to the criteria established for satisfactory performance below;

f. Monitor the performance of State Shellfish LEOs to ensure that the evaluation process is being performed consistent with NSSP requirements as described in the current NSSP *Guide for the Control of Molluscan Shellfish* and this guidance;

g. Maintain communication as needed with the Authority and other pertinent state officials, prospective and current State Shellfish LEOs and FDA Regional Shellfish Specialists relevant to the certification/recertification process;

h. Revoke certification of State Shellfish LEOs for cause; and,

i. Void certification when the need for a State Shellfish LEO no longer exists within the state shellfish sanitation program or when the State Shellfish LEO is no longer employed by the state.

~~Selection of State Shellfish LEOs should be based on the following criteria:~~

~~1.The individual must be administratively attached to a state central shellfish sanitation laboratory that has been found by the FDA to be in full conformance with NSSP requirements. To avoid the appearance of impropriety and maintain objectivity in the evaluation process, individuals certified as State Shellfish LEOs will not be allowed to evaluate their own laboratories. FDA will maintain the responsibility for evaluating these laboratories.~~

~~2.The individual must be an experienced analyst and should have laboratory supervision experience. To maintain the integrity of the evaluation process, this individual should not, however, have overall supervisory responsibilities for the laboratory or laboratories to be evaluated. If deemed necessary by an FDA Laboratory Evaluation Officer, the individual must conduct several laboratory evaluations jointly with the FDA Laboratory Evaluation Officer.~~

~~3.During the joint on-site laboratory evaluation with an FDA Laboratory Evaluation Officer, the individual must demonstrate competence in evaluating the laboratory's capability to support the NSSP. The evaluation will be performed and documented using the most current version of the applicable FDA Shellfish Laboratory Evaluation Checklist.~~

~~4 The individual must submit a written narrative report of the joint on-site evaluation to the FDA co-evaluator for review and comment. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in this evaluation write-up; and, where relevant an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations must be included in this write-up.~~

~~The FDA will issue a letter certifying each individual who successfully completes the certification process and will clear the evaluation report(s) for distribution to the laboratories evaluated with copies to the appropriate Shellfish Specialist.~~

~~Certification is normally effective for a period of three (3) years. Once certified, the individual is then expected to assume the following responsibilities:~~

State Shellfish Laboratory Evaluation Officer's Responsibilities

1. Conduct onsite laboratory evaluations at least every three (3) years. However, more frequent evaluations are strongly encouraged and may be ~~required~~ necessary with marginally performing laboratories, or when major changes in workloads or priorities have occurred or when there has been a substantial turnover of personnel, or, at the specific request of the Authority. ~~State Shellfish Control Authorities:~~
2. Provide appropriate post-evaluation follow-up for each laboratory evaluated;
3. Prepare timely narrative evaluation reports for all laboratories evaluated. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist for the component(s) evaluated and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in this narrative; and, where relevant, an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations should also be included in the narrative report. ~~Incorporating the requirements specified in 4 above;~~
4. Distribute completed evaluation reports with checklists to FDA and to the appropriate FDA Regional Shellfish Specialist.~~:~~
5. Inform ~~the appropriate~~ FDA Shellfish Laboratory Evaluation Officers when a laboratory has been found to be in nonconforming status.~~;~~
6. Coordinate proficiency testing at least yearly for all laboratories in the state supporting the microbiology component of the NSSP.
7. Prepare ~~at least~~ annually (in December) a summary list of ~~qualified analysts for each~~ all laboratories and qualified analysts within each laboratory by NSSP laboratory component supported ~~laboratory supporting the NSSP in the state~~ and transmit it to the ~~appropriate~~ FDA Shellfish Laboratory Evaluation Officers.

Certification Process

Certification is designed to be accomplished through individualized training and field standardization. Individuals are certified for evaluating either the microbiological and/or post harvest processing (PHP) and/or marine biotoxin components of the NSSP depending on their qualifications and the needs of the state shellfish sanitation program and at the discretion of FDA.

Field Standardization

1. Field standardization is designed to evaluate the prospective State Shellfish LEO's ability to determine the competence of the laboratory to meet NSSP laboratory requirements; recognize laboratory practices inconsistent with NSSP requirements when they occur; make appropriate recommendations for corrective action; and, provide the necessary follow-up activity to bring the laboratory into conformity with the NSSP.
2. Field standardization consists of one or several joint but independent onsite evaluations with an FDA Shellfish Laboratory Evaluation Officer and preparation of the corresponding narrative evaluation reports. The report(s) should consist of the completed FDA Shellfish Laboratory

Evaluation Checklist(s) and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in the narrative; and where relevant an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations should be included in this narrative report(s).

3. Field standardization should be performed in NSSP laboratories within the prospective State Shellfish LEO's home state to provide realistic evaluation scenarios. The narrative evaluation report detailing the evaluation findings must be prepared. The draft narrative report(s) with accompanying checklist(s) must be submitted to the certifying FDA Shellfish Laboratory Evaluation Officer within 60 days of the evaluation(s). All documents submitted will be reviewed for appropriate content, accuracy and uniformity of approach by the certifying FDA Shellfish Laboratory Evaluation Officer.
4. Field standardization is based on a pass fail system.

Certification

1. Certification is dependent upon the perspective State Shellfish LEO satisfying all the following performance criteria.
   a. Demonstration of good familiarity with evaluation requirements.
   b. Demonstration of a thorough knowledge of the evaluation methods and documents.
   c. Demonstration of the technical knowledge/familiarity with the analytical procedures being used.
   d. Ability to communicate effectively both orally and in writing.
   e. Successful completion of both training and field standardization.
2. Upon successful completion of the certification process, a letter of certification will be issued by the FDA Shellfish Laboratory Evaluation Officer and a copy will be sent to both the requesting Authority and the FDA Regional Shellfish Specialist.
3. Certification is normally valid for up to five (5) years unless revoked or voided.

Failure to be Certified

1. If a prospective State Shellfish LEO fails to satisfy any of the performance criteria listed above, he/she will not be certified.
2. As resources permit and at the discretion of FDA, the prospective State Shellfish LEO may receive additional training to better prepare him/her to be certified.
3. The requesting Authority may withdraw the prospective State Shellfish LEO from consideration.

Recertification

1. Recertification normally occurs every five (5) years and is contingent upon the continuing need in the state shellfish sanitation program for the services of a State Shellfish LEO.
2. Recertification is based on the State Shellfish LEO satisfactorily meeting the following employment and performance criteria.
   a. The individual must continue to be employed by the state and be free of any commercial, financial or other pressures or conflicts of interest real or perceived that may cause the State

Shellfish LEO to act in other than an impartial and non-discriminatory manner.

b. The individual must demonstrate continued competence in the evaluation of NSSP laboratories by performing one to several joint evaluations with an FDA Shellfish Laboratory Evaluation Officer and providing an appropriate narrative evaluation report to the FDA co-evaluator for review and comment for each of the laboratories jointly evaluated.

c. The individual must have performed laboratory evaluations at the minimum frequency prescribed in the current edition of the *Guide for the Control of Molluscan Shellfish* and have all Narrative evaluation reports up to date.

3. State Shellfish LEOs who successfully complete recertification will be issued a letter of recertification by FDA and be cleared to distribute the completed report(s) to the appropriate Regional Shellfish Specialist. A copy of this letter will be sent to the State Shellfish Control Authority and appropriate Regional Shellfish Specialist.

4. If FDA is unable to conduct a recertification visit by the expiration of the individual's certification, his/her certification may be extended until such time as recertification can be completed. If requested, a letter extending the certification can be provided as appropriate.

Revocation of Certification

1. State Shellfish LEO's who fail to meet any of the certification/recertification, employment or performance criteria listed above will have their certification revoked.
2. Certification may be voided when state shellfish sanitation programs no longer have a need for the services of a State Shellfish LEO.
3. Voided certifications may be reactivated at the discretion of FDA if the need for the analytical services of additional laboratories by the state shellfish sanitation program recurs.
4. Revoked certifications will not normally be restored.

~~Recertification of State Shellfish LEOs will normally occur triennially and will be based on satisfactorily meeting the following criteria:~~

~~1. The individual must continue to be administratively attached to a central state shellfish laboratory which is in full conformance with NSSP requirements;~~

~~2. The individual is not the supervisor of any of the laboratories to be evaluated;~~

~~3. The individual must demonstrate continued competence in evaluating the capability of laboratories to support the NSSP. If considered necessary, the individual will be required to performance to several joint evaluations with FDA Laboratory Evaluation Officer.~~

~~4. The individual must submit a written narrative report of the joint evaluation(s) to the FDA co-evaluator for review and comment. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist and the narrative portion should be prepared as above;~~

~~5. The individual must have all state laboratory evaluations, split sample(proficiency) test examinations, and reports current;~~

~~6. The individual should receive training as necessary, in laboratory~~

	~~evaluations and analytical procedures to remain proficient.~~ ~~State Shellfish LEOs who successfully complete this process will be issued a Letter of recertification by FDA and be cleared to distribute the evaluation reports to the laboratories evaluated with a copy to the appropriate Regional Shellfish Specialist. Normally recertification is effective for a period of three (3) years. Individuals who fail to meet the requirements for recertification will lose their certification until it is demonstrated that all requirements including adequate training are met.~~
Public Health Significance:	This guidance document is virtually unchanged since the inception of the program for utilizing State Shellfish Laboratory Evaluation Officers (State Shellfish LEOS) in the NSSP. This revised guidance updates and clarifies the process for selection, certification and recertification of State Shellfish LEOs.
Cost Information (if available):	NA
Action by 2013 Task Force I	Recommends referral of Proposal 13-117 to an appropriate committee as determined by the Conference Chairman.

Proposal Subject:	Dilution Guidance for Prohibited Zones Associated with Wastewater Discharges
Specific NSSP Guide Reference:	NSSP Guide Section IV. Guidance Documents Chapter II. Growing Areas
Text of Proposal/ Requested Action	US Food and Drug Administration requested that Task Force I consider the substitute language. .16 Determining Appropriately Sized Prohibited Areas Associated with Wastewater Treatment Plants Introduction Molluscan shellfish are filter feeders and therefore have the ability to concentrate microorganisms from the water column, including human pathogens and toxigenic micro-algae if these organisms are present. Concentrations of microorganisms in the shellfish may be as much as 100 times greater than those found in the water, and if the microorganisms are harmful to humans, illness can result. The correlation between sewage pollution of shellfish waters and illness has been demonstrated many times. Certain shellfish-borne infectious diseases are transmitted via the fecal-oral route, with the cycle beginning with the fecal contamination of the shellfish growing waters. In the winter of 1924-25, an oyster-borne typhoid outbreak occurred in the United States which caused a large number of illnesses and deaths (Lumsden, et al 1925). In response to this outbreak the National Shellfish Sanitation Program (NSSP) was initiated by the States, the U.S. Public Health Service, and the shellfish industry. Research at the time indicated that typhoid fever would not ordinarily be attributed to shellfish harvested from water in which not more than 50% percent of the one cc (ml) portions of water examined were positive for fecal coliform bacteria (an MPN of approximately 70 per 100 ml), provided that the areas were not subject to direct contamination with small amounts of fresh sewage which would not likely be revealed by routine bacteriological examination. As a result water quality criteria were established, namely; (1)The area be sufficiently removed from major sources of pollution so that the shellfish are not subjected to fecal contamination in quantities which might be dangerous to public health; (2)The area be free from pollution by even small quantities of fresh sewage; (3)Bacteriological examination does not ordinarily show the presence of the coli- aerogenes group of bacteria in one cc dilution of the growing area water. Once these standards were adopted in the United States in 1925, reliance on these criteria for evaluating the safety of shellfish harvesting areas has generally proven effective in preventing major outbreaks of disease transmitted by the fecal-oral route. Today, fecal and total coliforms are used as an index of the sanitary quality of a growing area and to foretell the possible presence of fecal transmitted bacterial pathogens. The goal of the NSSP remains the same – to ensure the safety of shellfish for human consumption by preventing

harvest from contaminated growing areas.

However, there is now ample scientific evidence to show that the current bacterial indicators are inadequate to predict the risk of viral illness for the following reasons:

(1) Enteric viruses are resistant to treatment and disinfection processes in a wastewater treatment plant (WWTP) and are frequently detected in t he WWTP's final effluent under normal operating conditions (Baggi et al. 2001; Burkhardt et al. 2005).

(2) Shellfish can bioaccumulate enteric viruses up to 100-fold from surrounding water (Seraichekas et al. 1968; Maalouf et al. 2011).

(3) Certain enteric viruses are retained by molluscan shellfish to a greater extent and for longer than the indicator bacteria currently used to classify shellfish growing areas (Sobsey et al. 1987; Dore & Lees 1995; Love et al. 2010). It has been well documented that enteric virus detection is not indexed by levels of conventional indicator bacteria.

For several decades now viral illnesses (in particular norovirus (NoV) and Hepatitis A (HAV)) have been the most common food safety problem associated with bivalve molluscan shellfish (Woods & Burkhardt. 2010; Iwamoto et al 2010; Scallan et al. 2011; Batz et al. 2012). NoV genogroups I, II and IV and HAV are human specific and transferred by the fecal-oral route. Because WWTPs do not completely remove infectious enteric viruses emphasis should be placed on the importance of ensuring there is adequate dilution between a sewage source and a shellfish growing area.
The purpose of this guidance is to provide the scientific basis and recommendations for determining appropriately sized Prohibited Areas (closure zones) based on the minimum criteria established under Section II, Chapter IV. @.03 E(5) of the Model Ordinance (Section E Prohibited Classification).

Classification Requirements for Growing Areas Associated with Waste Water Treatment Plants

The NSSP Model Ordinance (MO) requires that a comprehensive sanitary survey be undertaken prior to the classification of the growing area as Approved, Conditionally Approved, Restricted, or Conditionally Restricted.

The sanitary survey must take careful recognition of any WWTPs as they represent one of the major sources of human sewage pollution. It is preferable that the shellfish growing areas be sited so far away from sewage discharges that the WWTP effluent h as no hazardous effect, because there is a direct relationship between the level of WWTP effluent dilution and the level of enteric viruses detected in the shellfish (Goblick et al. 2011).

Delineation of the Prohibited Zone around a Wastewater Treatment Plant

The NSSP MO Section II, Chapter IV. @.03 (2) (b) states that all growing areas which have a sewage treatment plant outfall or other point source

outfall of public health significance within or adjacent to the shellfish growing area shall have a prohibited classification established adjacent to the outfall taking account of the following factors:

(1) The volume flow rate, location of discharge, performance of the wastewater treatment plant and the bacteriological or viral quality of the effluent;

(2) The decay rate of the contaminants of public health significance in the wastewater discharged;

(3) The wastewater's dispersion and dilution and the time of waste transport to the area where shellstock may be harvested; and

(4) The location of the shellfish resources, classification of adjacent waters and identifiable landmarks or boundaries.

There are several important considerations for the shellfish authority to consider when establishing the size of the prohibited zone:

(1) The distance to ensure that there is adequate dilution when the WWTP is operating as normal. "Normal" means that the WWTP is operating fully within the plant's design specifications, including design flows, treatment stages, disinfection, as well as compliance with all permit conditions.

If the plant is operating outside of the normal parameters it shall be considered to be malfunctioning.

(2) That the collection system has no malfunctions, bypasses or other factors that would lead to significant sewage leakages to the marine environment.

(3) That there is adequate time when any malfunction occurs to ensure that all harvesting ceases and closures are enforced, so that contaminated product does not reach the market.

The following guidelines shall be used when assessing these factors in the dilution analysis for the closure zone:

Volume flow rate: For a minimally sized prohibited zone for Conditionally Approved areas managed in part based on the performance of the WWTP, the maximum monthly average flow at the WWTP recorded in the Monthly Operating Reports (MORs) maintained by the WWTP permitting authority should be used considering at a minimum the most recent two years of flow records. If the maximum monthly average flow at the WWTP from two consecutive years of flow records is within 85 – 100% of the design flow, then the design flow should be used. Thus, these flow values are appropriate when establishing a minimally sized prohibited zone when the WWTP is considered to be operating under normal operating conditions.

Additional information and historical data may be accessed on the U.S. Environmental Protection Agency (EPA) website at: http://cfpub.epa.gov/dmr/index.cfm. Consistent with the EPA regulations in 40 CFR 122.2, the maximum monthly average flow, which is typically reported in the MOR, is defined as the average ''daily discharges'' over a calendar month, calculated as the sum of all '' daily discharges '' measured

during a calendar month divided by the number of ‘‘daily discharges’’ measured dur ing that month typically expressed in units of million gallons per day (MGD). Thus, the maximum monthly average flow is defined as the highest average monthly flow (MGD) within at a minimum the most recent consecutive two years of flow records. The design flow is defined as the flow (MGD) that the WWTP is designed to discharge and can be expressed as a daily, monthly, or annual discharge. In the design of WWTPs, various flow regimes are considered such as the average flow, maximum flow and peak (instantaneous) flow. However, it is important to note that certain tolerances are allowed under EPA NPDES program and WWTPs are not necessarily expected to meet permit conditions over all flow regimes. Thus, if permit limits are expressed as a monthly average it is considered acceptable for the permitted pollutants to exceed the permit on a short term basis as long as the permit condition (monthly average) is met. It is also important to note that EPA does not have any permit limitations established for the discharge of viruses.

In the context of public health, some of these flow regimes such as when average hourly flows exceed the design flow can be associated with periods of effluent degradation leading to an increase in the viral load in the effluent. Utilizing average hourly flows and comparing against the design flow ensures that the periods when effluent degradation are most likely to occur are adequately identified and assessed. Average hourly flow rates within the most recent two years of records should be evaluated to assess the likelihood that the average hourly flows can exceed the design flow. In the absence of supporting data, the conditional area should be closed when the average hourly flow rates exceed the WWTP design flow due to the potential degradation of the virological quality of treatment. FDA studies have determined that when WWTP average hourly flow rates exceed design flow the virological quality of effluent typically degrades beyond what is considered as normal treatment. Moreover, FDA bioaccumulation studies indicate that shellfish can accumulate significant levels of viral pathogens when exposed in durations of less than one hour. However, a flow level threshold above the design flow could be determined on a case by case basis provided the virological quality of the effluent is assessed. The average hourly flow is defined as the average flow measured over an hour. More detailed flow records are typically maintained and can be accessed through the permitted WWTP.

When conditional management based on WWTP performance is not employed the prohibited zone shall be sufficient in size to dilute the microbial loadings resulting from a WWTP malfunction (such as a sewage bypass or a loss of disinfection) to ensure the Approved area adjacent to the prohibited zone will meet the bacteriological standards for Approved area classification under all conditions including a WWTP malfunction. If the WWTP has no prior history of sewage bypasses then at a minimum a loss of disinfection malfunction shall be considered when sizing the prohibited zone. As many WWTP malfunctions occur from hydraulic overloading as a result of rainfall, snowmelt, storm events or periods of high flow, a maximum average hourly rate shall be considered when determining the size of the prohibited zone. The maximum average hourly flow is defined as the highest average hourly flow recorded within at a minimum) the most recent two consecutive years of flow records.

Location of discharge: The location of the discharge must be determined in order to define the distance from the point of effluent discharge to

shellfish growing areas that could be impacted. The distance from shore and the depth of the WWTP outfall also can be used in the dilution analysis of the discharge. The location of discharge includes the location, number, size and orientation of the discharge port(s) on the outfall or its diffuser.

When determining if a WWTP within the watershed or catchment area draining to a shellfish estuary potentially impacts a shellfish growing area, in the absence of a database collected, the NSSP recommends that a worst case raw sewage discharge be assumed. In this circumstance a level of 1.4×10^6 FC/100ml assumed for a raw sewage release-requires a 100,000:1 dilution to dilute the sewage sufficient to meet the approved area standard of 14 FC/100ml. If dilution analysis determines that the location of the discharge is such that the dilution of effluent would be greater than 100,000:1 then the WWTP could be considered located outside the zone of influence to the shellfish growing area. A lower dilution level could be justified provided that specific data to that particular WWTP demonstrates that a lower bacteriological level associated with a potential raw sewage discharge is supported. Additional or other site specific information also can be used to justify alternative approaches that may take into account other factors (such as no prior history of raw sewage discharges or containment structures sufficiently sized to accommodate a raw sewage event preventing a discharge).

It should also be noted that if shellfish harvesting occurs within the zone of influence from a WWTP then these areas are subject to a WWTP Management Plan as defined in Section II Chapter IV @. 03 C.(2)(a) of the MO. Additionally, if a departure of the normal WWTP function could potentially impact a shellfish growing area then the areas affected should be managed under a conditional management plan as defined in Section II Chapter IV @. 03 C.(2)(a) of the MO.

The minimum size of a prohibited zone for a conditional area under a WWTP management plan should be determined considering both the minimum dilution (1000:1) needed to mitigate the presence of viruses in treated effluent (or a scientifically based alternative approach) as well as the prerequisite notification time to close the conditional area during a WWTP malfunction or period of degraded effluent quality, prior to the conditional area receiving the impact from the WWTP effluent.

Performance of the WWTP: When considering the present and past performance of the WWTP, this review should include information regarding the wastewater collection system, inspection of essential plant components (including any monitoring and alarm systems), events whereby the plant exceeds its design capacity and an evaluation of the disinfection system.The plants past performance should also include a file review of the plant 's Discharge Monitoring Reports, considering at a minimum, the most recent two years of permit records.
When there is evidence that the WWTP exceeds design capacity, consideration should then be given to the frequency of such events and the effect this will have on the plant's ability to reduce the viral load of the effluent .

Consideration should also be given to the frequency of which the WWTP bypasses any stage of treatment or any condition that may degrade the quality of the effluent to determine the potential frequency a conditional growing area

may need to close over the course of a year. This assessment will determine the feasibility of operating a conditionally managed area based on WWTP performance.

Bacteriological or viral quality of the effluent: Discharge Monitoring Reports for WWTPs should be examined and periodically monitored to assess the reliability of the disinfection systems. Any samples collected to assess the reliability of the disinfection system should be collected during the period(s) of the year that the State Shellfish Control Authority (SSCA) deems most likely to experience adverse conditions in the treatment or disinfection processes that could affect effluent quality impacting receiving waters.

Results from any bacteriological or viral sampling and analyses must be correlated with WWTP operation and evaluated in terms of the minimum treatment expected when there is a malfunction, overloading or other poor operational condition. However, it is essential to recognize that water samples collected near discharge outfalls are not useful for determining the size of prohibited zones because normal operating conditions in WWTPs can effectively reduce or even eliminate the fecal and total coliforms - the current indicator microorganisms used to assess treatment efficiency. In contrast, many human enteric viruses are not inactivated by functional WWTP systems, hence the need for an adequate dilution zone between the outfall and the shellfish resource.

Decay rate of contaminants: It should be assumed that there is no fecal coliform or viral inactivation in the effluent during possible upset conditions in the WWTP. There are a number of conditions that affect bacterial and viral inactivation, including temperature, exposure to sunlight and sedimentation levels in the water (Burkhardt et al, 2000; Lees, 2002; LaBelle, 1980; Griffen, 2003). Scientists are unsure how long viruses remain viable in the marine environment, but it is likely to be weeks or months (Younger, 2002), and enteroviruses have been found in marine sediments suggesting that these sediments can be a source upon resuspension (Lewis, 1986). Moreover, molluscan shellfish have been found to retain viruses to a greater extent and for much longer periods than they do bacteria (Sobsey et al, 1987; Richards, 1988; Dore and Lees, 1995; Dore et al, 2000; Shieh et al, 2000).

Waste water dispersion and dilution: Dispersion of the effluent refers to the spread, location, and shape of the discharge plume with time as it leaves the WWTP outfall. Dilution of the effluent refers to the amount of receiving water that is entrained within a particular time or distance from the outfall, e.g. the dilution of the effluent within the time or distance it takes to reach the border of the prohibited zone. A dye study can be used to measure the dilution and dispersion of the effluent during specific discharge conditions. Computer modeling programs can also be used to estimate the dispersion and dilution of the effluent plume from WWTPs.

In poorly flushed estuaries and coastal embayments there is the potential for WWTP effluent build-up that further reduces the availability of “clean” waters to both dilute contaminant loadings and purge shellfish of contaminants (Goblick et al., 2011).

Time of waste transport to the shellfish harvest site: When there is a WWTP malfunction it is important that adequate systems are in place to officially

close the harvest area before the effluent impacts the shellfish. This is a mandatory requirement for conditional management of shellfish harvest areas and all parties must agree in writing on the process steps necessary to close the harvest area after such events. Both time of travel and dilution should be considered when sizing a prohibited zone around a WWTP outfall adjacent to a conditional growing area. The overall sizing of the prohibitive zone should satisfy both a minimum dilution of 1000:1 and also factor in adequate time to respond to a malfunction event. When establishing the time of travel between the WWTP and the classified area, consideration should be given to the worst scenarios which would cause the fastest travel. For example, the peak current flows at or near the outfall during ebb tide and flood tide to determine effluent transport speeds. Current velocity information may need to be generated if such information is not available or adequate for the area of the outfall. Current velocity information can be obtained from hydrographic dye studies, drogue studies, or current meter data conducted in the vicinity of the outfall.

Location of shellfish resources: The best information that is available should be used for locating shellfish resources near the outfall. Subtidal shellfish resources may also be identified in sanitary surveys near WWTP outfalls. Therefore the SSCA must establish closure zones at WWTP outfalls in accordance with the classification requirements of the Model Ordinance.

Classification of Adjacent Waters: If the SSCA's dilution analysis determines that the shellfish water quality standards for approved waters are met at the boundary of the prohibited area during potential upset conditions, the shellfish area adjacent to the prohibited area need not be classified as Conditionally Approved and may be classified as Approved.

Scientific Rationale for 1000:1 Dilution Guidance

Since 1987 FDA has recommended at training courses and other venues the use of a 1000:1 dilution as the minimum level of dilution needed around a WWTP outfall to mitigate the impact of viruses for shellfish harvest areas managed conditionally based on the performance of the WWTP. It has been advised that conditional management based on WWTP performance may not be appropriate for all WWTP's that are located within proximity to shellfish harvest areas and recommended only for large, highly efficient WWTPs that are well monitored.. In 1995 this estimated level of necessary dilution was further calculated and explained by FDA using assumptions based on the most relevant scientific literature available at that time (Kohn, et al. 1995; Havelaar et al. 1993; Kapikian et al. 1990; Liu et al. 1966). Since then major advances in the detection and enumeration of NoV in wastewater and shellfish have been made, and advances in fluorometer technologies have enabled more sophisticated hydrographic dye study methods. Using these advances, FDA has conducted dye studies supplemented with the testing of shellfish sentinels for enteric viruses and their surrogates. This has afforded FDA for the first time with a means to directly determine the viral risk posed by WWTP effluent on shellfish resources. During recent years FDA has presented the findings from these studies at regional shellfish meetings, at the biennial ISSC meeting, at international scientific conferences and to international partners engaged in collaborative projects. Results from these studies are referred to herein as part of the scientific basis for the current recommended guidance.

	In 2008 FDA performed an investigation in the upper portion of Mobile Bay, Alabama, the results of which were published in the Journal of Shellfish Research (Goblick, et al., 2011). The article describes how FDA used the aforementioned technical advances to prospectively assess the 1995 1000:1 dilution estimate recommendation and determine if this level of dilution is appropriate to mitigate the risk of viruses discharged in treated wastewater effluent. From 2008 through 2012 FDA conducted four additional studies (Hampton Roads, Virginia; Yarmouth, Maine; Coos Bay, Oregon; Blaine, Washington). In each of these studies, FDA evaluated male-specific coliphage (MSC) and NoV levels in shellfish together with the dilutions of WWTP effluent. The studies were designed to build a more comprehensive and in- depth understanding of viral impacts posed by WWTPs on shellfish resources. To date, findings from these studies demonstrate that achieving a steady-state 1000:1 dilution level in the requisite Prohibited area appears to be adequate for mitigating the impacts of viruses on shellfish when WWTPs have typical treatment and disinfection practices, such as secondary treatment and the use of chlorine, and when they are operating under normal conditions. Results further indicate that in certain instances, such as when WWTPs begin to exceed their design capacity, bypass treatment, or otherwise malfunction, the 1000:1 dilution level may be inadequate and emergency closure procedures should be considered within the conditional area management plan. Under such circumstances, conditional area management plans should ensure there is sufficient time for notification to the State Shellfish Control Authority (SSCA) and for subsequent notifications closing the conditional area to harvesting. MSC results in shellfish from the 2008-2012 studies were evaluated using 50 PFU/100 g as the threshold level of concern for MSC, since this is the level under the Model Ordinance (Section II, Chapter IV, @.03 A(5)(c)(ii)) used for re-opening harvest areas after an emergency closure due to raw untreated sewage discharged from a large community sewage collection system or a WWTP. For conventional WWTPs operating under normal conditions, there were at least four occasions when dilution levels were between 700:1 and 1000:1 and MSC levels in shellfish exceeded 50 PFU/100g, but there were no occasions in which MSC levels exceeded 50 PFU/100g and dilution was greater than 1000:1. For conventional WWTPs operating under malfunction conditions, such as when flow rates exceeded the design capacity or during a treatment stage bypass, MSC levels in shellfish exceeded 50 PFU/100g in at least 13 instances in which dilution was greater than 1000:1. When evaluating the NoV results of the 2008 – 2012 studies FDA used a value of 300 RT-PCR units of NoV/100 gram of digestive gland (digestive diverticula) as the threshold. This value was considered significant since at this level shellfish related illnesses have been reported and demonstrated by the analysis of meal remnants. In examining the results from all the studies, there were no cases in which conventional WWTPs operating under normal conditions produced results greater than 300 NoV particles/100 g of DD in oyster sentinels when dilution levels at the associated sentinel stations were greater than 1000:1. When dilution levels were less than 1000:1, levels of NoV GII greater than 300 NoV particles/100 g of DD were detected, and on one occasion around 8000 NoV

	particles/100g DD were found. On three occasions during which WWTPs were operating under malfunction conditions (as previously described), thirteen (13) oyster samples were found with NoV GII levels greater than 300 NoV particles/100 g DD when dilution was close to or greater than 1000:1. These results emphasize the critical need for sufficient notification time, meaning travel time from the WWTP discharge in Prohibited Area is long enough to close the shellfish growing area in the event of a malfunction. This preventative measure may necessitate the Prohibited Area be larger than the zone necessary to achieve 1000:1 dilution. In one instance, an unconventional WWTP that used membrane filtration technology rather than conventional treatment with chlorine or UV disinfection was assessed. The levels of NoV GII in shellfish sentinels near this WWTP were greater than 300 NoV particles/100 g of DD, even when dilution levels were greater than 1000:1, and on two occasions when dilution levels exceeded 10,000:1. In seven (7) instances, NoV levels at the plant were greater than 300 NoV particles/100g of DD. MSC levels were similarly high, with all six (6) samples tested having MSC levels greater than 800 PFU/100g, and in one sample greater than 10,000 PFU/100g, even though dilution levels were higher than 1000:1. This analysis demonstrates the need to assess WWTPs with unique treatment systems on a case by case basis, since some may perform better than conventional WWTPs at removing viruses and some may perform significantly worse. The overall results of FDA's studies demonstrate a strong relationship between increased levels of enteric viruses and MSC and decreased levels of dilution. This trend was observed in all of the studies conducted by FDA at conventional WWTPs. The FDA studies also suggested that certain factors, such as the quality of sewage treatment or the time of year, may exert influences on the levels of viruses discharged and hence the minimum level of dilution needed to ensure shellfish safety. However, at this time FDA does not have reliable data to justify a recommended minimum dilution less than 1000:1 or to establish any variable dilution thresholds corresponding to and dependent on such factors. It is recognized that these criteria could be determined by a State Shellfish Control Authority (SSCA) on a case by case basis, where factors of WWTP performance, disinfection method, tidal flushing, and seasonal impacts may vary. These and other factors that might influence virus levels in the shellfish can be considered by SSCAs when assessing how best to manage conditional growing areas based on WWTP performance. Using dilution levels lower than 1000:1 or other alternative approaches for managing the viral risk posed by WWTP effluents are cited in Alternate Options section (see below). However, when there is insufficient information available for a growing area to support the use of a lower level of dilution, the 1000:1 dilution should be employed. Alternate Options It is expected that the principles of this guidance shall be followed to ensure compliance with the dilution requirements of the Model Ordinance. An alternative minimum waste water dilution threshold value may be appropriate for situations in which highly effective WWTP facilities reduce the viral load of the effluent, or seasonal or geographical factors reduce the risk of viral contamination at the shellfish growing area. Alternative options for calculating the size of the prohibited zone to mitigate the virological effects of WWTP

discharges at the shellfish growing area may be used provided that they are based on sound scientific principles that can be verified. For example, it is reasonable to expect a potentially higher reduction in viral load from a properly maintained wastewater treatment system employing ultraviolet (UV) disinfection with tertiary treatment operating under optimum design flow conditions. Regardless of the technology employed any proposed alternative minimum threshold would need validation. MSC could potentially be used on a case- by-case basis as the validation process (for example to validate treatment efficiency) if demonstrated it is a successful/feasible strategy for the given location/situation

It should be noted that any alternate approach would need to consider the time of waste transport to the shellfish harvest site. As described in this guidance in geographic regions with large tidal amplitudes and/or swift tidal currents, the time of waste transport to the shellfish harvest site may be the determining factor in sizing the prohibited zone. However, there may be various strategies that could be employed to address the time of waste transport to the shellfish harvest site. For example, it may be reasonable to expect that if a facility utilized a sufficiently sized containment structure (such as the equivalent to 24-hour holding for the design capacity of the plant) in the event of a malfunction, this would allow the SSCA additional time to react to the event and take any necessary precautions. Regardless of technology or best management practices employed any proposed alternative strategy would need to be validated (ie verifying that a containment structure is properly sized and working effectively).

There are likely other alternatives in addressing the potential impact of wastewater on shellfish growing areas and approaches in validating these options. However, the flex ibi lit y rem ains with the SSCA's to determine the appropriate alternate option and validation process that can be verified.

References

Batz, M. B., Hoffman, S., Morris, G.J. Ranking the Disease Burden of 14 Pathogens in Food Sources in the United States Using Attribution Data from Outbreak Investigations and Expert Elicitation. Journal of Food Protection, Vol 75 (7):1278- 1291

Baggi, F., A. Demarta, and R. Peduzzi. (2001) Persistence of viral pathogens and bacteriophages during sewage treatment: lack of correlation with indicator bacteria. Res. Microbiol. 152, 743–751

Bedford, A.J., G. Williams, and A.R. Bellamy. 1978. Virus accumulation by the rock oyster *Crassostrea glomerata*. Appl. Environ Microbiol. 35(6):1012-8.

Brooks, N.H. 1960. Diffusion of sewage effluent in an ocean current. In: E.A. Pearson, editor. Proceedings of the First Conference on Waste Disposal in the Marine Environment. New York, NY: Pergamon Press. pp. 246-267.

Burkhardt, W. III, J.W. Woods, and K.R. Calci. 2005. Evaluation of Wastewater Treatment Plant Efficiency to Reduce Bacterial and Viral Loading Using Real-time RT-PCR. Poster Presentation, ASM, Atlanta, GA, Annual Educational Conference.

Burkhardt, W. III, W.D. Watkins, and S.R. Rippey. 1992. Seasonal effects on accumulation of microbial indicator organisms by *Mercenaria mercenaria*. Appl. Environ. Microbiol. 58:826-31.

Burkhardt, W. III, and K.R. Calci. 2000. Selective accumulation may account for shellfish-associated viral illness. Appl. Environ. Microbiol., Vol. 66(4): 1375-1378.

Burkhardt, W III. Calci, K. R., Watkins, W. D., Rippey S. R. and Chirtel, S. J. 2000. Inactivation of Indicator Organisms in Estaurine Waters. Wat. Res. 34(9): 2207- 2214.

DePaola, A., J.L. Jones, J. Woods, W. Burkhardt III, K.R. Calci, J.A. Krantz, J.C. Bowers, K. Kasturi, R.H. Byars, E. Jacobs, D. Williams-Hill, & K. Nabe. 2010. Bacterial and Viral Pathogens in Live Oysters: 2007 United States Market Survey. Appl. Environ. Microbiol. 76(9):2754-2768.

Dore, W.J. and D.N. Lees. 1995. Behavior of *Escherichia coli* and male-specific bacteriophage in environmentally contaminated bivalve molluscs before and after depuration. Appl. Environ. Microbiol. 61:2830-2834.

Dore, W.J., K. Henshilwood, and D.N. Lees. 2000. Evaluation of F-Specific RNA bacteriophage as a candidate human enteric virus indicator for bivalve molluscan shellfish. Appl. Environ. Microbiol. 66(4):1280-1285.

Enriquez, R., G.G. Frosner, V. Hochstein-Mintzel, S. Riedermann, and G. Reinhardt. 1992. Accumulation and persistence of hepatitis A virus in mussels. J. Med Virol. 37(3):174-9.

Goblick, G.N., Anbarchian J M,. Woods J.,, Burkhardt W. and Calci K. 2011. Evaluating the Dilution of Wastewater Treatment Plant Effluent and Viral Impacts on Shellfish Growing Areas in Mobile Bay, Alabama. Journal of Shellfish Research, Vol. 30 (3), 1-9.

Havelaar, AH, M. van Olphen, and Y.C. Drost.1993.F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. Appl. Environ. Microbiol. 59(9):2956-2962.

Iwamoto, M., Ayers, T., Mahon, B and Swerdlow, D.L 2010. Epidemiology of Seafood-Associated Infections in the USA. Clinical Microbiology Reveiws. April,2010 . p399-411.

Jaykus, L., M.T. Hemard, and M.D. Sobsey. 1994. Human enteric pathogenic viruses. In: C.R. Hackney and M.D. Pierson, editors. Environmental Indicators and Shellfish Safety. New York, NY: Chapman and Hall. pp. 289-330.

Kapikian, AZ and Chanock RM. 1990. Norwalk Group of Virus in Virology. New York, NY: Raven Press Ltd. pp. 671-693.

Kohn, et al. 1995. An Outbreak of Norwalk Virus Gastroenteritis Associated with Eating Raw Oysters, Implications of Maintaining Safe Oyster Beds. JAMA.

Lumsden, L.L. Hassetline, H. E., Leake, J.P. and Veldee, M. V. A Typhoid Fever Epidemic Caused by Oyster-Borne Infection (1924-5). Supplement No

	50 to the Public Health Reports. Washington Government Printing Office 1925. Liu, OC, Seraichekas, HR, Murphy, BL. 1966. Viral Pollution of Shellfish, I: Some Basic Facts of Uptake. Proc. Soc. Exp. Biol. Med. 123:481-487. Love, D.C., Lovelace, G.L., & Sobsey, M.D. 2010. Removal of *Escherichia coli, Enterococcus fecalis,* coliphage MS2, poliovirus, and hepatitis A virus from oysters (*Crassostrea virginica*) and hard shell clams (*Mercinaria mercinaria*) by depuration. *Int.J.Food Microbiol.*, 143, (3) 211-217 Maalouf, F. Schaeffer, J., Parnaudeau, S., Le Pendu, J.. Atmar, R., Crawford, S.E. & Le Guyader, F.S. (2011) Strain-dependent Norovirus bioaccumulation in oysters. *Applied and Environmental Microbiology* 77(10): 3189 Metcalf, T.G., B. Mullin, D. Eckerson, E. Moulton, and E.P. Larkin. 1979. Accumulation and depuration of enteroviruses by the soft-shelled clam, *Mya arenaria*. Appl. Environ. Microbiol. 38(2):275-82. Nash, J.D. 1995. Buoyant Discharges into Reversing Ambient Currents, MS Thesis, DeFrees Hydraulics Laboratory, Cornell University, Ithaca, NY. Scallan, E., Hoekstra, R.M. Tauxe, R. V et al. Foodborne Illness Acquired in the United States – Major Pathogens. Emerging Infectious Diseases Vol17. No1, January 2011. www.cdc.gov/eld Seraichekas, H. R., D. A. Brashear, J. A. Barnick, P. F. Carey & O. C. Liu. 1968. Viral deputation by assaying individual shellfish. Appl. Microbiol. 16:1865-1871. Shieh, C. Y., R.S. Baric, J.W. Woods, and K.R. Calci. 2003. Molecular surveillance of enterovirus and Norwalk-like virus in oysters relocated to a municipal-sewage- impacted Gulf estuary. Appl. Environ. Microbiol. 69(12):7130-7136. Sobsey, M.D., A.L. Davis, and V.A. Rullman. 1987. Persistence of hepatitis A virus and other viruses in depurated eastern oysters. In: NOAA, editor. Proceedings, Oceans ' 87. Halifax, Nova Scotia: NOAA. 5:1740-1745.
Public Health Significance:	The public health purpose of this guidance is to provide the scientific basis and recommendations for determining appropriately sized Prohibited Areas (closure zones) around waste water treatment plants (WWTP). Section II, Chapter IV @ .03 (5) currently mandates that a prohibited zone be established, but there is no specific guidance information on how to calculate the size of the prohibited zone to ensure that microbiological pathogens (particularly viruses) from WWTP do not adversely impact the growing area at the time of harvest. It is expected that this guidance will provide all ISSC stakeholders with better information on which to make informed, scientifically based decisions
Cost Information (if available):	

Action by 2013 Task Force I	Recommends referral of Proposal 13-118 to an appropriate committee as determined by the Conference Chairman with additional instructions to the ISSC Executive Office to create a workgroup to meet quarterly and report back to the Conferenc at the next ISSC meeting.

Proposal Subject:	Revisions to Chapter III. Requirements for the Authority
Specific NSSP Guide Reference:	2011 NSSP Guide Section II. Model Ordinance Chapter III. Laboratory
Text of Proposal/ Requested Action	**@.02 Methods.** A. Microbiological. Methods for the analyses of shellfish and shellfish growing or harvest waters shall be: (1) The Approved NSSP Methods validated for use in the National Shellfish Sanitation Program under Procedure XVI. of the Constitution, Bylaws and Procedures of the ISSC and ~~/ or~~ cited in the Guidance Documents Chapter II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests. (2) When there is an immediate or ongoing critical need for a method and no Approved NSSP Method exists, the following may be used: (a) A validated AOAC, BAM, or EPA method; (b) An Emergency Use Method pursuant to .02 D. (1) and (2) below. B. Chemical and Physical. Methods for the analyses of shellfish and shellfish harvest waters shall be: (1) The Approved NSSP Methods validated for use in the National Shellfish Sanitation Program under Procedure XVI. Of the Constitution, Bylaws and Procedures of the ISSC and cited in the Guidance Documents Chapter II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests. ~~Methods for the analysis of shellfish and shellfish growing or harvest waters shall:~~ ~~▪ Be the current AOAC or APHA method for all physical and chemical measurements; and~~ ~~▪ Express results of all chemical and physical measurements in standard units, and not instrument readings.~~ (2) Results shall be expressed for chemical and physical measurements in standard units and not instrument readings. ~~(2)~~(3) When there is an immediate or ongoing critical need for a Method and no Approved NSSP Method exists, the following may be used: (a) A validated AOAC, BAM, or EPA method; (b) An Emergency Use Method pursuant to .02 D. (1) and (2) below. C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be: (1) The Approved NSSP Methods validated for use in the national Shellfish Sanitation Program under Procedure XVI. Of the Constitution, Bylaws and Procedures of the ISSC and cited in the Guidance Documents Chpater II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests. ~~The current AOAC and APHA methods used in the bioassay for paralytic shellfish poisoning toxins; and~~ ~~(2) The current APHA method used in the bioassay for *Karenia brevis* toxins; or~~ ~~(3) Approved NSSP Methods validated for use under Procedure XVI. of the Constitution, Bylaws and Procedures of the ISSC and/or cited in the Guidance Documents Chapter II. Growing Areas .11 Approved~~

	~~National Shellfish Sanitation Program Laboratory Tests.~~ ~~(4)~~(2) When there is an immediate or ongoing critical need for a method and no Approved NSSP Method exists, the following may be used: (a) A validated AOAC, BAM, or EPA method; (b) An Emergency Use Method pursuant to .02 D. (1) and (2) below. D. Emergency Use Methods. (1) When there is an immediate or critical need and no Approved NSSP Method exists, an unapproved or non-validated method may be used for a specific purpose provided that: (a) The appropriate FDA Regional Office is notified within a reasonable period of time regarding the method employed; and (b) The ISSC Executive Board is notified within a reasonable period of time regarding the method employed. (2) When it is necessary to continue the use of the emergency method employed under D. (1) beyond the initial critical need, then the following minimum criteria shall be provided to the ISSC Executive Board for interim approval: (a) Name of Method. (b) Date of Submission. (c) Specific purpose or intent of the method for use in the NSSP. (d) Step by step procedure including equipment, reagents and safety requirements necessary to run the method. (e) Data generated in the development and/or trials of the method and/or comparing to approved methods if applicable. (f) Any peer reviewed articles detailing the method. (g) Name of developer(s) or Shellfish Control Authority submitter. (h) Developer/submitter contact information. (3) Within two (2) years of Executive Board interim approval of the Emergency Use Method, the entire Single Lab Validation Protocol should be submitted. The Laboratory Methods Review Committee will report to the Executive Board on the status of the Single Lab Validation Protocol data submission.
Public Health Significance:	This revision to Chapter III. Laboratory is necessary to clarify and guide users to the location within the Guidance Documents that lists the approved NSSP laboratory tests in .11 Approved NSSP Laboratory Tests. All approved laboratory tests are now listed in Table .11 Approved NSSP Laboratory Tests with the Guidance Document.
Cost Information (if available):	
Action by 2013 Task Force	Recommends adoption of Proposal 13-119-L as amended. **@.02 Methods.** A. Microbiological. Methods for the analyses of shellfish and shellfish growing or harvest waters shall be: (1) The Approved NSSP Methods validated for use in the National Shellfish Sanitation Program under Procedure XVI. of the Constitution, Bylaws and Procedures of the ISSC and/or cited in the Guidance Documents Chapter II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests.

(2) When there is an immediate or ongoing critical need for a method and no Approved NSSP Method exists, the following may be used:

(a) A validated AOAC, BAM, or EPA method;

(b) An Emergency Use Method pursuant to .02 D. (1) and (2) below.

B. Chemical and Physical. Methods for the analyses of shellfish and shellfish harvest waters shall be:

(1) The Approved NSSP Methods validated for use in the National Shellfish Sanitation Program under Procedure XVI. Of the Constitution, Bylaws and Procedures of the ISSC and/or cited in the Guidance Documents Chapter II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests.

(2) Results shall be expressed for chemical and physical measurements in standard units and not instrument readings.

(3) When there is an immediate or ongoing critical need for a Method and no Approved NSSP Method exists, the following may be used:

(a) A validated AOAC, BAM, or EPA method;

(b) An Emergency Use Method pursuant to .02 D. (1) and (2) below.

C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be:

(1) The Approved NSSP Methods validated for use in the national Shellfish Sanitation Program under Procedure XVI. Of the Constitution, Bylaws and Procedures of the ISSC and/or cited in the Guidance Documents Chapter II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests.

(2) When there is an immediate or ongoing critical need for a method and no Approved NSSP Method exists, the following may be used:

(a) A validated AOAC, BAM, or EPA method;

(b) An Emergency Use Method pursuant to .02 D. (1) and (2) below.

D. Emergency Use Methods.

(1) When there is an immediate or critical need and no Approved NSSP Method exists, an unapproved or non-validated method may be used for a specific purpose provided that:

(a) The appropriate FDA Regional Office is notified within a reasonable period of time regarding the method employed; and

(b) The ISSC Executive Board is notified within a reasonable period of time regarding the method employed.

(2) When it is necessary to continue the use of the emergency method employed under D. (1) beyond the initial critical need, then the following minimum criteria shall be provided to the ISSC Executive Board for interim approval:

(a) Name of Method.

(b) Date of Submission.

(c) Specific purpose or intent of the method for use in the NSSP.

(d) Step by step procedure including equipment, reagents and safety requirements necessary to run the method.

(e) Data generated in the development and/or trials of the method and/or comparing to approved methods if applicable.

	(f) Any peer reviewed articles detailing the method. (g) Name of developer(s) or Shellfish Control Authority submitter. (h) Developer/submitter contact information. (3) Within two (2) years of Executive Board interim approval of the Emergency Use Method, the entire Single Lab Validation Protocol should be submitted. The Laboratory Methods Review Committee will report to the Executive Board on the status of the Single Lab Validation Protocol data submission.

Proposal Subject:	Male-specific Coliphage Method for Quahogs (M. mercenaria)
Specific NSSP Guide Reference:	NSSP Guide Section IV Guidance Documents Chapter II Growing Areas .11 Approved Limited Use Methods for Microbiological Testing
Text of Proposal/ Requested Action	This submission presents the 'Male-specific Coliphage method for Quahogs (M. mercenaria)' for consideration as an approved limited use method for microbiological testing. At the 2009 ISSC, the 'Modified Double Agar Overlay Method for Determining Male-specific Coliphage in Soft-shelled Clams and American Oysters' was accepted as an approved limited use method for microbiological testing for re-opening growing areas after emergency closures due to sewage spills. SLV work with quahogs has demonstrated comparable performance characteristics as with soft-shelled clams and American oysters. The requested action is to include quahogs in the footnote for MSC along with soft-shelled clams and American oysters in NSSP Guide Section IV Guidance Documents Chapter II Growing Areas .11 Approved Limited Use Methods for Microbiological Testing.
Public Health Significance:	The MSC method for quahogs was used recently by the State of New Jersey to re-open growing areas after the devastating effects of Superstorm Sandy. Increasingly, enumeration of male-specific coliphage (MSC) in soft-shelled clams, American oysters, and quahogs is needed in the NSSP to assess *viral* contamination in molluscan shellfish harvested from growing areas where fecal coliform levels in both water quality and shellfish meats may be misleading. MSC is a specialized indicator of *viral* sewage contamination, which is substantially more meaningful than fecal coliform or E. coli in evaluating the safety of shellstock harvested from growing areas potentially impacted by treated and partially treated wastewater.
Cost Information (if available):	This method for the enumeration of male-specific coliphage in soft-shelled clams, American oysters, and quahogs is inexpensive, easy to perform, and rapid, providing results within 24 hours. The cost of laboratory glassware, plastic-ware, agars, and reagents is approximately $25 per shellfish sample. In a well-equipped laboratory, the method requires 6 hours of time from initiating host to pouring plates. Hands on technician time to perform this test is significantly less on the order of 1-4 hours per test depending upon how many tests are done per day. The most expensive piece of equipment is a refrigerated centrifuge plus rotor, which costs approximately $12,000. There are no special skill sets required beyond those required to operate a state-approved shellfish laboratory under the NSSP.
Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended adoption of this method for use in detecting MSC in hard clams and direct the Executive Office to amend the table at Section IV. Chapter 2 @ .11 to add Quahogs to footnote #1
***Action by 2013 Task Force I**	Recommends adoption of Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-120-L